WEST Search History

DATE: Wednesday, July 16, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L10	early secretory antigenic target	3	L10
L9	L8 and ifn$	28	L9
L8	L7 and t cell	32	L8
L7	L6 and (assay or elisa or elispot or diagno$)	32	L7
L6	l4 and epitope	32	L6
L5	L4 and es1	0	L5
L4	L3 and tuberculosis	51	L4
L3	esat-6	51	L3
L2	pathan-ansar.in.	0	L2
L1	lalvani-ajit.in.	6	L1

END OF SEARCH HISTORY

09/838839

Welcome to STN International! Enter x:x

LOGINID:ssspta1813nxm

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	Feb 24	PCTGEN now available on STN
NEWS 4	Feb 24	TEMA now available on STN
NEWS 5	Feb 26	NTIS now allows simultaneous left and right truncation
NEWS 6	Feb 26	PCTFULL now contains images
NEWS 7	Mar 04	SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 8	Mar 24	PATDPAFULL now available on STN
NEWS 9	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 10	Apr 11	Display formats in DGENE enhanced
NEWS 11	Apr 14	MEDLINE Reload
NEWS 12	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 13	Jun 13	Indexing from 1947 to 1956 added to records in CA/CAPLUS
NEWS 14	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 15	Apr 28	RDISCLOSURE now available on STN
NEWS 16	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 17	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 18	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 19	May 19	Simultaneous left and right truncation added to WSCA
NEWS 20	May 19	RAPRA enhanced with new search field, simultaneous left and right truncation
NEWS 21	Jun 06	Simultaneous left and right truncation added to CBNB
NEWS 22	Jun 06	PASCAL enhanced with additional data
NEWS 23	Jun 20	2003 edition of the FSTA Thesaurus is now available
NEWS 24	Jun 25	HSDB has been reloaded
NEWS 25	Jul 16	Data from 1960-1976 added to RDISCLOSURE

NEWS EXPRESS	April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

* * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

```
FILE 'HOME' ENTERED AT 12:28:34 ON 17 JUL 2003

=> file biosis medline agricola embase caba wpids  japio biotechds lifesci caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'BIOSIS' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'MEDLINE' ENTERED AT 12:28:50 ON 17 JUL 2003

FILE 'AGRICOLA' ENTERED AT 12:28:50 ON 17 JUL 2003

FILE 'EMBASE' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 Elsevier Science B.V.  All rights reserved.

FILE 'CABA' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 CAB INTERNATIONAL (CABI)

FILE 'WPIDS' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 THOMSON DERWENT

FILE 'JAPIO' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 Japanese Patent Office (JPO)- JAPIO

FILE 'BIOTECHDS' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 THOMSON DERWENT AND INSTITUTE FOR SCIENTIFIC INFORMATION

FILE 'LIFESCI' ENTERED AT 12:28:50 ON 17 JUL 2003
COPYRIGHT (C) 2003 Cambridge Scientific Abstracts (CSA)


FILE 'CAPLUS' ENTERED AT 12:28:50 ON 17 JUL 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

=> e lalvani ajit/au
E1          111     LALVANI A/AU
E2            1     LALVANI A M/AU
E3           40 --> LALVANI AJIT/AU
E4            1     LALVANI B H/AU
E5            1     LALVANI D/AU
E6            2     LALVANI D D/AU
E7            1     LALVANI G H/AU
E8           13     LALVANI H/AU
E9            1     LALVANI HARESH/AU
E10           3     LALVANI K/AU
E11           1     LALVANI K S/AU
E12           1     LALVANI K SINGH/AU

=> s e1-e3
L1          152 ("LALVANI A"/AU OR "LALVANI A M"/AU OR "LALVANI AJIT"/AU)

=> e pathan ansar/au
E1            7     PATHAN A S/AU
E2            6     PATHAN A Z/AU
E3            4 --> PATHAN ANSAR/AU
E4           15     PATHAN ANSAR A/AU
E5            1     PATHAN ANSAR AHMED/AU
E6            8     PATHAN ASAD/AU
E7            3     PATHAN ASAD Z/AU
```

```
E8            1      PATHAN AYAZ/AU
E9            2      PATHAN D I/AU
E10          10      PATHAN E/AU
E11           1      PATHAN E M/AU
E12           8      PATHAN F/AU

=> s e1-e5
L2           33 ("PATHAN A S"/AU OR "PATHAN A Z"/AU OR "PATHAN ANSAR"/AU OR
                "PATHAN ANSAR A"/AU OR "PATHAN ANSAR AHMED"/AU)

=> s l1-l2
L3          169 (L1 OR L2)

=> s l3 and esat-6
L4           38 L3 AND ESAT-6

=> s l4 and (es1 or es2 or es3)
L5            0 L4 AND (ES1 OR ES2 OR ES3)

=> dup rem l4
PROCESSING COMPLETED FOR L4
L6           12 DUP REM L4 (26 DUPLICATES REMOVED)

=> d bib ab 1-12

L6   ANSWER 1 OF 12  WPIDS (C) 2003 THOMSON DERWENT    DUPLICATE 1
AN   2002-583633 [62]   WPIDS
DNN  N2002-462811       DNC C2002-165046
TI   Determining the progress of a mycobacterial infection, by direct ex vivo
     quantitation of ESAT-6-specific T cells.
DC   B04 D16 S03
IN   LALVANI, A
PA   (ISIS-N) ISIS INNOVATION LTD
CYC  100
PI   WO 2002054072 A2 20020711 (200262)* EN   53p
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM
            ZW
ADT  WO 2002054072 A2 WO 2002-GB55 20020108
PRAI US 2001-259868P  20010108; GB 2001-432      20010108
AB   WO 200254072 A UPAB: 20020926
     NOVELTY - Determining (M1) the efficacy of treatment for mycobacterial
     infection, involves determining the level of T cells specific for a
     mycobacterial antigen that has decreased after the treatment and therefore
     determining the efficacy of the treatment.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:
          (1) an agent (I) which prevents or treats mycobacterial infection in
     the manufacture of a medicament for the treatment of an individual in whom
     a treatment using a therapeutic substance has been found to have low
     efficacy using (M1), where the agent is different from the therapeutic
     substance using (M1) and for the manufacture of a medicament for the
     treatment of an individual who has been found to have a latent infection
     using (M2);
          (2) determining (M2) the presence of a latent infection in an
     individual, determining in a sample from the individual for the presence
     of T cells specific for a mycobacterial antigen;
          (3) an agent (II) which prevents or treats mycobacterial infection in
     the manufacture for the treatment of an individual who has been found to
     have a latent infection using (M2);
```

(4) determining (M3) the effect of an intervention on a mycobacterial infection in an individual, involves measuring the effect on the levels of T cells in samples from the individual and therefore determining the effect of the intervention;

(5) treating (M4) an individual infected by a mycobacterium, involves administering to an individual in whom treatment using a therapeutic substance has been found to have low efficacy using (M1), an agent which prevents or treats mycobacterial infection, where the agent is different from the therapeutic substance; and

(6) treating (M5) an individual infected by a mycobacterium by administering to an individual who has been found to have a latent infection using (M2), an agent which prevents or treats mycobacterial infection.

ACTIVITY - Antibacterial.

No suitable data given.

MECHANISM OF ACTION - None given.

USE - (M1) is useful for determining the efficacy of treatment for mycobacterial infection, the mycobacterial infection is Mycobacterium tuberculosis or M.bovis infection. (M2) is useful for determining the presence of a latent infection in a sample from the individual for the presence of T cells specific for a mycobacterial antigen. (M3) is useful for determining the effect of an intervention on a mycobacterial infection in an individual. (M4) is useful for treating an individual infected by a mycobacterium. (I) and (II) are useful for manufacturing a medicament for treating or preventing mycobacterial infection (claimed).

Dwg.0/8

L6 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2002:716868 CAPLUS
DN 137:246533
TI Mycobacterium tuberculosis epitopes in vaccines and detection of mycobacterial-specific cytotoxic T-cells
IN **Lalvani, Ajit**; **Pathan, Ansar A.**; Hill, Adrian V. S.
PA UK
SO U.S. Pat. Appl. Publ., 19 pp., Cont.-in-part of U.S. Ser. No. 467,893, abandoned.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002131976	A1	20020919	US 2001-916201	20010727
PRAI	US 1998-113783P	P	19981223		
	US 1999-467893	B2	19991221		

AB A method of detecting an anti-mycobacterial CD8 T cell response comprising contacting a population of CD8 T cells of an individual with one or more peptides selected from the peptides represented by SEQ ID NO: 3, 4, 7, 8, 9, 10, 11 or 12, and, optionally, one or two further peptides represented by SEQ ID NO: 1 and/or 2, wherein one or more of said peptides may be substituted by an analog which binds a T cell receptor which recognizes the corresponding substituted peptide, and detg. whether CD8 T cells of the CD8 T cell population recognize the peptide(s). The invention also provides a method of vaccinating against infection by a mycobacterium, wherein the vaccination leads to a CD8 T cell response, comprising administering (i) a CD8 T cell epitope of a mycobacterium protein, (ii) an analog of the epitope which is capable of inhibiting the binding of the epitope to a T cell receptor, (iii) a precursor or (i) or (ii) which is capable of being processed to provide (i) or (ii), or (iv) a polynucleotide which is capable of being expressed to provide (i), (ii) or (iii). The method of detecting CD8 T cells is an ELISPOT assay which detects interferon-.gamma., released by the T cells following peptide recognition, using an immobilized anti-IFN-.gamma. antibody.

L6 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2003:141590 BIOSIS
DN PREV200300141590
TI Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of Mycobacterium tuberculosis-specific T cells.
AU Chapman, Ann L. N.; Munkanta, Mwansa; Wilkinson, Katalin A.; **Pathan, Ansar A.**; Ewer, Katie; Ayles, Helen; Reece, William H.; Mwinga, Alwyn; Godfrey-Faussett, Peter; **Lalvani, Ajit (1)**
CS (1) Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Level 7, Oxford, OX3 9DU, UK: ajit.lalvani@ndm.ox.ac.uk UK
SO AIDS (Hagerstown), (22 November 2002) Vol. 16, No. 17, pp. 2285-2293. print.
ISSN: 0269-9370.
DT Article
LA English
AB Objectives: An accurate test for Mycobacterium tuberculosis infection is urgently needed. The tuberculin skin test (TST) lacks sensitivity, particularly in HIV-infected individuals, and has poor specificity because of antigenic cross-reactivity with Bacillus Calmette-Guerin (BCG) vaccination. **ESAT-6** and CFP-10 are antigens expressed in M. tuberculosis, but not in Mycobacterium bovis BCG and most environmental mycobacteria. We investigated whether T cells specific for these antigens could serve as accurate markers of M. tuberculosis infection in an area of high tuberculosis and HIV prevalence. Methods: Using the rapid ex-vivo enzyme-linked immunospot (ELISPOT) assay for IFN-gamma, we enumerated T cells specific for **ESAT-6**, CFP-10 and purified protein derivative (PPD) in blood samples from 50 Zambian tuberculosis patients, 75 healthy Zambian adults, and 40 healthy UK residents. TSTs were performed in 49 healthy Zambian adults. Results: All (100%; n=11) and 90% (n=39) of HIV-negative and HIV-positive tuberculosis patients, respectively, had detectable **ESAT-6**- or CFP-10-specific T cells. The **ESAT-6** /CFP-10-based ELISPOT assay was positive in 37 out of 54 HIV-negative healthy Zambians, suggesting a 69% prevalence of latent M. tuberculosis infection. Fewer HIV-positive Zambians possessed **ESAT-6** /CFP-10-specific T cells, but the impact of HIV infection was less on this assay than on the PPD-based ELISPOT or TST. Conclusion: The **ESAT-6**/CFP-10-based ELISPOT assay detects active tuberculosis in HIV-positive individuals with high sensitivity. It is more specific, and possibly more sensitive, than PPD-based methods of detecting latent M. tuberculosis infection, and may potentially improve the targeting of isoniazid preventative therapy to HIV-positive individuals with latent tuberculosis infection.

L6 ANSWER 4 OF 12 MEDLINE DUPLICATE 3
AN 2001567381 MEDLINE
DN 21528960 PubMed ID: 11673535
TI Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment.
AU Pathan A A; Wilkinson K A; Klenerman P; McShane H; Davidson R N; Pasvol G; Hill A V; **Lalvani A**
CS Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.
SO JOURNAL OF IMMUNOLOGY, (2001 Nov 1) 167 (9) 5217-25.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Abridged Index Medicus Journals; Priority Journals
EM 200112
ED Entered STN: 20011024
Last Updated on STN: 20020122
Entered Medline: 20011205
AB The wide spectrum of clinical outcomes following infection with Mycobacterium tuberculosis is largely determined by the host immune response; therefore, we studied several clinically defined groups of individuals (n = 120) that differ in their ability to contain the bacillus. To quantitate M. tuberculosis-specific T cells directly ex vivo, we enumerated IFN-gamma-secreting CD4 T cells specific for **ESAT-6**, a secreted Ag that is highly specific for M. tuberculosis, and a target of protective immune responses in animal models. We found that frequencies of circulating **ESAT-6** peptide-specific IFN-gamma-secreting CD4 T cells were higher in latently infected healthy contacts and subjects with minimal disease and low bacterial burdens than in patients with culture-positive active pulmonary tuberculosis ($p = 0.009$ and $p = 0.002$, respectively). Importantly, the frequency of these Ag-specific CD4 T cells fell progressively in all groups with treatment ($p = 0.005$), suggesting that the lower responses in patients with more extensive disease were not due to tuberculosis-induced immune suppression. This population of M. tuberculosis Ag-specific Th1-type CD4 T cells appears to correlate with clinical phenotype and declines during successful therapy; these features are consistent with a role for these T cells in the containment of M. tuberculosis in vivo. Such findings may assist in the design and evaluation of novel tuberculosis vaccine candidates.

L6 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:359931 BIOSIS
DN PREV200100359931
TI Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells.
AU **Lalvani, Ajit (1)**; **Pathan, Ansar A.**; Durkan, Helen; Wilkinson, Katalin A.; Whelan, Adam; Deeks, Jonathan J.; Reece, William H. H.; Latif, Mohammed; Pasvol, Geoffrey; Hill, Adrian V. S.
CS (1) Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU: ajit.lalvani@ndm.ox.ac.uk UK
SO Lancet (North American Edition), (23 June, 2001) Vol. 357, No. 9273, pp. 2017-2021. print.
ISSN: 0099-5355.
DT Article
LA English
SL English
AB Background: Identification of individuals latently infected with Mycobacterium tuberculosis is an important part of tuberculosis control. The current method, the tuberculin skin test (TST), has poor specificity because of the antigenic cross-reactivity of purified protein derivative (PPD) with M bovis BCG vaccine and environmental mycobacteria. **ESAT-6** is a secreted antigen that is highly specific for M tuberculosis complex, but is absent from M bovis BCG. With an enzyme-linked immunospot (ELISPOT) assay for interferon gamma, we have identified **ESAT-6**-specific T cells as an accurate marker of M tuberculosis infection. Methods: We did a prospective, masked study of 50 healthy contacts, with varying but well defined degrees of exposure to M tuberculosis, who attended an urban contact-tracing clinic. We assessed and compared the efficacy of our assay and TST for detection of symptomless infected individuals by correlation of test results with the degree of exposure to an infectious index case. Findings: The **ESAT-6** ELISPOT assay results had a strong positive relation with increasing intensity of exposure (odds ratio=9.0 per unit increase in level of exposure (95% CI 2.6-31.6), p=0.001), whereas TST

either alone or in combination, is new.

DETAILED DESCRIPTION - Novel method of diagnosing infection in a host (M), or exposure of a host, to a mycobacterium which expresses **ESAT-6**, comprises contacting T cells from the host with at least 1 of 11 peptides ((I)-(XI)) of 15 amino acids (aa), or their analogues which bind a T cell receptor that binds (I)-(XI), but not peptides (III) or (V) (or their analogues) either alone or in combination. E.g.:

Met-Thr-Glu-Gln-Gln-Trp-Asn-Phe-Ala-Gly-Ile-Glu-Ala-Ala-Ala (I);
Gln-Lys-Trp-Asp-Ala-Thr-Ala-Thr-Glu-Leu-Asn-Asn-Ala-Leu-Gln (III);
Asn-Leu-Ala-Arg-Thr-Ile-Ser-Glu-Ala-Gly-Gln-Ala-Met-Ala-Ser (V);
Glu-Gly-Lys-Gln-Ser-Leu-Thr-Lys-Leu-Ala-Ala-Ala-Trp-Gly-Gly (VII);
Asn-Val-Thr-Ser-Ile-His-Ser-Leu-Leu-Asp-Glu-Gly-Lys-Gln-Ser (IX);

and

Thr-Ala-Thr-Glu-Leu-Asn-Asn-Ala-Leu-Gln-Asn-Leu-Ala-Arg-Thr (XI).

INDEPENDENT CLAIMS are also included for the following:

(1) a kit for carrying out (M) comprising at least 1 of (I)-(XI) or their analogues, and optionally a means for detecting the recognition of the peptide by the T cell;

(2) a peptide as in (I)-(XI);

(3) a diagnostic product or panel as in (M); and

(4) a polynucleotide capable of expressing at least 1 of peptide or analogue as in (M) and/or (2).

USE - The methods and kits are useful for diagnosing micobacterial (especially Mycobacterium tuberculosis or M. bovis) infection, optionally in vivo (claimed). The peptides or their analogues may also be used to produce antibodies specific for the peptide (claimed).

ADVANTAGE - Tests using the novel peptides will not give a false positive results (indicating infection or exposure to a mycobacterium) for patients vaccinated with BCG.

Dwg.0/0

L6 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8

AN 2000:453913 BIOSIS

DN PREV200000453913

TI High frequencies of circulating IFN-gamma-secreting CD8 cytotoxic T cells specific for a novel MHC class I-restricted Mycobacterium tuberculosis epitope in M. tuberculosis-infected subjects without disease.

AU **Pathan, Ansar A.**; Wilkinson, Katalin A.; Wilkinson, Robert J.; Latif, Mohammed; McShane, Helen; Pasvol, Geoffrey; Hill, Adrian V. S.; **Lalvani, Ajit (1)**

CS (1) Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Level 7, Oxford, OX3 9DU UK

SO European Journal of Immunology, (September, 2000) Vol. 30, No. 9, pp. 2713-2721. print.
ISSN: 0014-2980.

DT Article

LA English

SL English

AB MHC class I-restricted CD8 cytotoxic T lymphocytes (CTL) are essential for protective immunity to Mycobacterium tuberculosis in animal models but their role in humans remains unclear. We therefore studied subjects who had successfully contained M. tuberculosis infection in vivo, i. e. exposed healthy household contacts and individuals with inactive self-healed pulmonary tuberculosis. Using the ELISPOT assay for IFN-gamma, we screened peptides from **ESAT-6**, a secreted antigen that is highly specific for M. tuberculosis. We identified a novel nonamer epitope: unstimulated peripheral blood-derived CD8 T cells displayed peptide-specific IFN-gamma release ex vivo while CD8 T cell lines and clones exhibited HLA-A68.02-restricted cytolytic activity and recognized endogenously processed antigen. The frequency of CD8 CTL specific for this single M. tuberculosis epitope, 1/2500 peripheral blood lymphocytes, was

equivalent to the combined frequency of all IFN-gamma-secreting purified protein derivative-reactive T cells ex vivo. This highly focused CTL response was maintained in an asymptomatic contact over 2 years and is the most potent antigen-specific anti-mycobacterial CD8 CTL response hitherto described. Thus, human M. tuberculosis-specific CD8 CTL are not necessarily associated with active disease per se. Rather, our results are consistent with a protective role for these **ESAT-6** -specific CD8 T cells in the long-term control of M. tuberculosis in vivo in humans.

L6 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1999:21646 BIOSIS
DN PREV199900021646
TI Cytotoxic T-lymphocytes against malaria and tuberculosis: From natural immunity to vaccine design.
AU **Lalvani, Ajit (1)**; Hill, Adrian V. S.
CS (1) Nuffield Dep. Clin. Med., Inst. Mol. Med., Univ. Oxford, John Radcliffe Hosp., Oxford OX3 9DU UK
SO Clinical Science (London), (Nov., 1998) Vol. 95, No. 5, pp. 531-538. ISSN: 0143-5221.
DT Article
LA English
AB 1. Mycobacterium tuberculosis and the liver stage of Plasmodium falciparum are intracellular pathogens which are potentially susceptible to cytotoxic T-lymphocytes, a crucial component of the protective immune response to viral infections. Evidence from animal models points to a protective role for cytotoxic T-lymphocytes against M. tuberculosis and P. falciparum, but cytotoxic T-lymphocytes specific for these pathogens have been difficult to identify in man. 2. Using a reverse immunogenetic approach, candidate epitopes from selected antigens of P. falciparum and M. tuberculosis were used to detect peptide-specific cytotoxic T-lymphocyte responses in individuals exposed to these pathogens. Cytotoxic T-lymphocyte activity was detected by the 51Cr release cytotoxicity assay and a sensitive ELISPOT assay for single-cell interferon-y release. 3. In naturally exposed, partially immune Africans in The Gambia, eight largely conserved cytotoxic T-lymphocyte epitopes in P. falciparum, restricted by several different HLA class I alleles, were identified. Several epitopes were also recognized in Tanzanians and cytotoxic T-lymphocytes recognized endogenously processed antigen. 4. In tuberculosis patients with HLA-852, a CD8+ cytotoxic T-lymphocyte epitope was identified in **ESAT-6**, a secreted antigen specific for M. tuberculosis complex but absent in BCG. Cytotoxic T-lymphocytes exhibited HLA-B52-restricted peptide-specific interferon-gamma release and lytic activity and recognized endogenously processed antigen. S. These studies demonstrate that CD8+ cytotoxic T-lymphocytes specific for mycobacterial and protozoal antigens are induced during natural infections in humans. The identification of these T-cells endorses current strategies to develop cytotoxic T-lymphocyte-inducing vaccines against P. falciparum and M. tuberculosis and highlights candidate antigens for inclusion in subunit vaccines.

L6 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:131124 BIOSIS
DN PREV199900131124
TI Identification of conserved, CD8+ cytotoxic T cell epitopes in **ESAT-6**, a tuberculosis vaccine candidate.
AU Pathan, A. (1); Brookes, R. (1); Pritchard, H. (1); Wilkinson, R.; Pasvol, G.; Hill, A. (1); **Lalvani, A. (1)**
CS (1) Nuffield Dep. Clin. Med., John Radcliffe Hosp., Oxford UK
SO Immunology, (Dec., 1998) Vol. 95, No. SUPPL. 1, pp. 108.
Meeting Info.: 6th Annual Congress of the British Society for Immunology Harrogate, England, UK December 1-4, 1998

ISSN: 0019-2805.
DT Conference
LA English

L6 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:112697 BIOSIS
DN PREV199900112697
TI Human T cell responses to the antigen **ESAT-6** characterize a vaccine candidate and potential diagnostic test for tuberculosis.
AU Pathan, A. (1); Brookes, R. (1); Pritchard, H. (1); Wilkinson, R.; Pasvol, G.; Hill, A. (1); **Lalvani, A. (1)**
CS (1) Nuffield Dep. Clinical Med., John Radcliffe Hosp., Oxford UK
SO Immunology, (Dec., 1998) Vol. 95, No. SUPPL. 1, pp. 90.
Meeting Info.: 6th Annual Congress of the British Society for Immunology Harrogate, England, UK December 1-4, 1998
ISSN: 0019-2805.
DT Conference
LA English

=> s esat-6
L7 583 ESAT-6

=> s l7 and tuberculosis
L8 564 L7 AND TUBERCULOSIS

=> s l8 and t cell recogni?
L9 11 L8 AND T CELL RECOGNI?

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 3 DUP REM L9 (8 DUPLICATES REMOVED)

=> d bib ab 1-3

L10 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2002:559608 BIOSIS
DN PREV200200559608
TI Epitope mapping of the immunodominant antigen TB10.4 and the two homologous proteins TB10.3 and TB12.9, which constitute a subfamily of the **esat-6** gene family.
AU Skjot, Rikke Louise Vinther; Brock, Inger; Arend, Sandra M.; Munk, Martin E.; Theisen, Michael; Ottenhoff, Tom H. M.; Andersen, Peter (1)
CS (1) Department of TB Immunology, Statens Serum Institut, Artillerivej 5, DK-2300, Copenhagen S: pa@ssi.dk Denmark
SO Infection and Immunity, (October, 2002) Vol. 70, No. 10, pp. 5446-5453. print.
ISSN: 0019-9567.
DT Article
LA English
AB The human **T-cell recognition** of the low-molecular-mass culture filtrate antigen TB10.4 was evaluated in detail. The molecule was strongly recognized by T cells isolated from **tuberculosis** (TB) patients and from BCG-vaccinated donors. The epitopes on TB10.4 were mapped with overlapping peptides and found to be distributed throughout the molecule. The broadest response was found in TB patients, whereas the response in BCG-vaccinated donors was focused mainly toward a dominant epitope located in the N terminus (amino acids 1 to 18). The gene encoding TB10.4 was found to belong to a subfamily within the **esat-6** family that consists of the three highly homologous proteins TB10.4, TB10.3, and TB12.9 (Rv0288, Rv3019c, and Rv3017c, respectively). Southern blot analysis combined with database

groups of possible relevance for vaccine development. The study population consisted of 65 human immunodeficiency virus-negative donors from the Hossana Regional Hospital, Hossana, Ethiopia. Peripheral blood leukocytes from the donors were stimulated with different antigens and immune responses were determined. Household contacts produced significantly higher levels of gamma interferon (IFN-gamma) than the TB patients in response to antigens present in ST-CF and the 10 narrow-molecular-mass fractions. A similar difference in leukocyte proliferative responses to the antigens between the two groups was also found. In general, while all fractions stimulated immune responses, the highest activity was seen with the low-molecular-mass fractions, which include well-defined TB antigens such as **ESAT-6**. Leukocytes from contacts of TB patients with severe disease produced higher levels of antigen-specific IFN-gamma than those from contacts of patients with minimal disease. Both groups of contacts exhibited higher cell-mediated responses than the patients themselves. The enhanced immune response of healthy contacts, especially those of patients with severe disease, to secreted mycobacterial antigens is suggestive of an early stage of infection by M. **tuberculosis**, which could in time result in overt disease or containment of the infection. This possibility is currently being investigated by follow-up studies of the household contacts.

```
=> d his

     (FILE 'HOME' ENTERED AT 12:28:34 ON 17 JUL 2003)

     FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,
     LIFESCI, CAPLUS' ENTERED AT 12:28:50 ON 17 JUL 2003
                E LALVANI AJIT/AU
L1          152 S E1-E3
                E PATHAN ANSAR/AU
L2           33 S E1-E5
L3          169 S L1-L2
L4           38 S L3 AND ESAT-6
L5            0 S L4 AND (ES1 OR ES2 OR ES3)
L6           12 DUP REM L4 (26 DUPLICATES REMOVED)
L7          583 S ESAT-6
L8          564 S L7 AND TUBERCULOSIS
L9           11 S L8 AND T CELL RECOGNI?
L10           3 DUP REM L9 (8 DUPLICATES REMOVED)

=> s l8 and t cell (5a) recogni?
L11         35 L8 AND T CELL (5A) RECOGNI?

=> s l11 and (diagnosis or diagnostic or assay or detect?)
   6 FILES SEARCHED...
L12         20 L11 AND (DIAGNOSIS OR DIAGNOSTIC OR ASSAY OR DETECT?)

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           7 DUP REM L12 (13 DUPLICATES REMOVED)

=> d bib ab 1-7

L13  ANSWER 1 OF 7  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN   2003:223665  BIOSIS
DN   PREV200300223665
TI   Recognition of mycobacterial epitopes by T cells across mammalian species
     and use of a program that predicts human HLA-DR binding peptides to
     predict bovine epitopes.
AU   Vordermeier, Martin (1); Whelan, Adam O.; Hewinson, R. Glyn
CS   (1) TB Research Group, Veterinary Laboratories Agency-Weybridge, New Haw,
```

amongst the HLA molecules involved in the presentation of **ESAT-6** and its peptides to human Th1 cells. In addition, the T-cell lines were cytotoxic for monocytes and macrophages pulsed with **ESAT-6** and peptides. In conclusion, the recognition of **ESAT-6** by IFN-gamma-secreting and cytotoxic CD4+ T cells in association with frequently expressed HLA class II molecules supports the application of this antigen to either specific **diagnosis** or subunit vaccine design.

L13 ANSWER 3 OF 7 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-583633 [62] WPIDS
DNN N2002-462811 DNC C2002-165046
TI Determining the progress of a mycobacterial infection, by direct ex vivo quantitation of **ESAT-6**-specific T cells.
DC B04 D16 S03
IN LALVANI, A
PA (ISIS-N) ISIS INNOVATION LTD
CYC 100
PI WO 2002054072 A2 20020711 (200262)* EN 53p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
ADT WO 2002054072 A2 WO 2002-GB55 20020108
PRAI US 2001-259868P 20010108; GB 2001-432 20010108
AB WO 200254072 A UPAB: 20020926
NOVELTY - Determining (M1) the efficacy of treatment for mycobacterial infection, involves determining the level of T cells specific for a mycobacterial antigen that has decreased after the treatment and therefore determining the efficacy of the treatment.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:

(1) an agent (I) which prevents or treats mycobacterial infection in the manufacture of a medicament for the treatment of an individual in whom a treatment using a therapeutic substance has been found to have low efficacy using (M1), where the agent is different from the therapeutic substance using (M1) and for the manufacture of a medicament for the treatment of an individual who has been found to have a latent infection using (M2);

(2) determining (M2) the presence of a latent infection in an individual, determining in a sample from the individual for the presence of T cells specific for a mycobacterial antigen;

(3) an agent (II) which prevents or treats mycobacterial infection in the manufacture for the treatment of an individual who has been found to have a latent infection using (M2);

(4) determining (M3) the effect of an intervention on a mycobacterial infection in an individual, involves measuring the effect on the levels of T cells in samples from the individual and therefore determining the effect of the intervention;

(5) treating (M4) an individual infected by a mycobacterium, involves administering to an individual in whom treatment using a therapeutic substance has been found to have low efficacy using (M1), an agent which prevents or treats mycobacterial infection, where the agent is different from the therapeutic substance; and

(6) treating (M5) an individual infected by a mycobacterium by administering to an individual who has been found to have a latent infection using (M2), an agent which prevents or treats mycobacterial infection.

ACTIVITY - Antibacterial.
No suitable data given.
MECHANISM OF ACTION - None given.

USE - (M1) is useful for determining the efficacy of treatment for mycobacterial infection, the mycobacterial infection is Mycobacterium **tuberculosis** or M.bovis infection. (M2) is useful for determining the presence of a latent infection in a sample from the individual for the presence of T cells specific for a mycobacterial antigen. (M3) is useful for determining the effect of an intervention on a mycobacterial infection in an individual. (M4) is useful for treating an individual infected by a mycobacterium. (I) and (II) are useful for manufacturing a medicament for treating or preventing mycobacterial infection (claimed). Dwg.0/8

L13 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2003 ACS
AN 2002:716868 CAPLUS
DN 137:246533
TI Mycobacterium **tuberculosis** epitopes in vaccines and **detection** of mycobacterial-specific cytotoxic T-cells
IN Lalvani, Ajit; Pathan, Ansar A.; Hill, Adrian V. S.
PA UK
SO U.S. Pat. Appl. Publ., 19 pp., Cont.-in-part of U.S. Ser. No. 467,893, abandoned.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002131976	A1	20020919	US 2001-916201	20010727
PRAI US 1998-113783P	P	19981223		
US 1999-467893	B2	19991221		

AB A method of **detecting** an anti-mycobacterial CD8 T cell response comprising contacting a population of CD8 T cells of an individual with one or more peptides selected from the peptides represented by SEQ ID NO: 3, 4, 7, 8, 9, 10, 11 or 12, and, optionally, one or two further peptides represented by SEQ ID NO: 1 and/or 2, wherein one or more of said peptides may be substituted by an analog which binds a **T cell** receptor which **recognizes** the corresponding substituted peptide, and detg. whether CD8 T cells of the CD8 **T cell** population **recognize** the peptide(s). The invention also provides a method of vaccinating against infection by a mycobacterium, wherein the vaccination leads to a CD8 T cell response, comprising administering (i) a CD8 T cell epitope of a mycobacterium protein, (ii) an analog of the epitope which is capable of inhibiting the binding of the epitope to a T cell receptor, (iii) a precursor or (i) or (ii) which is capable of being processed to provide (i) or (ii), or (iv) a polynucleotide which is capable of being expressed to provide (i), (ii) or (iii). The method of **detecting** CD8 T cells is an ELISPOT **assay** which **detects** interferon-.gamma., released by the T cells following peptide recognition, using an immobilized anti-IFN-.gamma. antibody.

L13 ANSWER 5 OF 7 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-365579 [31] WPIDS
DNN N2000-273545 DNC C2000-110441
TI Novel method of diagnosing infection, or exposure of a host, to a mycobacterium comprising contacting T cells from the host with **ESAT-6** derived peptides.
DC B04 D16 S03
IN LALVANI, A; PATHAN, A A; AJIT, L; ANSAR, A P
PA (ISIS-N) ISIS INNOVATION LTD
CYC 91
PI WO 2000026248 A2 20000511 (200031)* EN 33p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 9964809 A 20000522 (200040)
BR 9915055 A 20010807 (200152)
EP 1144447 A2 20011017 (200169) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI
ZA 2001003356 A 20020327 (200230) 51p
CN 1350546 A 20020522 (200258)
JP 2002532064 W 20021002 (200279) 48p

ADT WO 2000026248 A2 WO 1999-GB3635 19991103; AU 9964809 A AU 1999-64809 19991103; BR 9915055 A BR 1999-15055 19991103, WO 1999-GB3635 19991103; EP 1144447 A2 EP 1999-952697 19991103, WO 1999-GB3635 19991103; ZA 2001003356 A ZA 2001-3356 20010425; CN 1350546 A CN 1999-813005 19991103; JP 2002532064 W WO 1999-GB3635 19991103, JP 2000-579635 19991103

FDT AU 9964809 A Based on WO 200026248; BR 9915055 A Based on WO 200026248; EP 1144447 A2 Based on WO 200026248; JP 2002532064 W Based on WO 200026248

PRAI US 1998-107004P 19981104; GB 1998-24213 19981104

AB WO 200026248 A UPAB: 20000630

NOVELTY - Diagnosing infection in a host (M), or exposure of a host, to a mycobacterium which expresses **ESAT-6**, comprises contacting T cells from the host with at least 1 of 11 peptides ((I)-(XI)) of 15 amino acids (aa), or their analogues which bind a T cell receptor that binds (I)-(XI), but not peptides (III) or (V) (or their analogues) either alone or in combination, is new.

DETAILED DESCRIPTION - Novel method of diagnosing infection in a host (M), or exposure of a host, to a mycobacterium which expresses **ESAT-6**, comprises contacting T cells from the host with at least 1 of 11 peptides ((I)-(XI)) of 15 amino acids (aa), or their analogues which bind a T cell receptor that binds (I)-(XI), but not peptides (III) or (V) (or their analogues) either alone or in combination. E.g.:

Met-Thr-Glu-Gln-Gln-Trp-Asn-Phe-Ala-Gly-Ile-Glu-Ala-Ala-Ala (I);
Gln-Lys-Trp-Asp-Ala-Thr-Ala-Thr-Glu-Leu-Asn²Asn-Ala-Leu-Gln (III);
Asn-Leu-Ala-Arg-Thr-Ile-Ser-Glu-Ala-Gly-Gln-Ala-Met-Ala-Ser (V);
Glu-Gly-Lys-Gln-Ser-Leu-Thr-Lys-Leu-Ala-Ala-Ala-Trp-Gly-Gly (VII);
Asn-Val-Thr-Ser-Ile-His-Ser-Leu-Leu-Asp-Glu-Gly-Lys-Gln-Ser (IX);

and

Thr-Ala-Thr-Glu-Leu-Asn-Asn-Ala-Leu-Gln-Asn-Leu-Ala-Arg-Thr (XI).

INDEPENDENT CLAIMS are also included for the following:

(1) a kit for carrying out (M) comprising at least 1 of (I)-(XI) or their analogues, and optionally a means for **detecting** the **recognition** of the peptide by the **T cell**;

(2) a peptide as in (I)-(XI);

(3) a **diagnostic** product or panel as in (M); and

(4) a polynucleotide capable of expressing at least 1 of peptide or analogue as in (M) and/or (2).

USE - The methods and kits are useful for diagnosing micobacterial (especially Mycobacterium **tuberculosis** or M. bovis) infection, optionally in vivo (claimed). The peptides or their analogues may also be used to produce antibodies specific for the peptide (claimed).

ADVANTAGE - Tests using the novel peptides will not give a false positive results (indicating infection or exposure to a mycobacterium) for patients vaccinated with BCG.

Dwg.0/0

L13 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2003 ACS
AN 2000:282543 CAPLUS
DN 133:41846
TI Antigen specificity in experimental bovine **tuberculosis**
AU Rhodes, S. G.; Gavier-Widen, D.; Buddle, B. M.; Whelan, A. O.; Singh, M.;

```
=> s l8 and vaccin?
L14         382 L8 AND VACCIN?

=> d his

     (FILE 'HOME' ENTERED AT 12:28:34 ON 17 JUL 2003)

     FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,
     LIFESCI, CAPLUS' ENTERED AT 12:28:50 ON 17 JUL 2003
                E LALVANI AJIT/AU
L1          152 S E1-E3
                E PATHAN ANSAR/AU
L2           33 S E1-E5
L3          169 S L1-L2
L4           38 S L3 AND ESAT-6
L5            0 S L4 AND (ES1 OR ES2 OR ES3)
L6           12 DUP REM L4 (26 DUPLICATES REMOVED)
L7          583 S ESAT-6
L8          564 S L7 AND TUBERCULOSIS
L9           11 S L8 AND T CELL RECOGNI?
L10           3 DUP REM L9 (8 DUPLICATES REMOVED)
L11          35 S L8 AND T CELL (5A) RECOGNI?
L12          20 S L11 AND (DIAGNOSIS OR DIAGNOSTIC OR ASSAY OR DETECT?)
L13           7 DUP REM L12 (13 DUPLICATES REMOVED)
L14         382 S L8 AND VACCIN?

=> s l14 and (treating or treatment or preventing or prevention)
L15          69 L14 AND (TREATING OR TREATMENT OR PREVENTING OR PREVENTION)

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16          47 DUP REM L15 (22 DUPLICATES REMOVED)

=> d bib ab 1-47

L16  ANSWER 1 OF 47  CAPLUS  COPYRIGHT 2003 ACS
AN   2003:174232  CAPLUS
DN   138:220358
TI   Avirulent pathogenic micro-organisms over-expressing microbial homologous
     antigens in the development of vaccine
IN   Schurig, Gerhardt; Boyle, Stephen M.; Sriranganathan, Nammalwar
PA   USA
SO   U.S. Pat. Appl. Publ., 15 pp., Cont.-in-part of U.S. Ser. No. 692,621.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 2
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   US 2003044431      A1   20030306       US 2002-268673   20021011
     WO 9929340         A1   19990617       WO 1997-US23032  19971205
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR,
             KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG,
             US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,
             GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,
             GN, ML, MR, NE, SN, TD, TG
     US 6149920         A    20001121       US 1998-91521    19980619
PRAI WO 1997-US23032    W    19971205
```

US 1998-91521 A3 19980619
US 2000-692621 A2 20001020

AB This invention relates to an over-expressing homologous antigen **vaccine**, a method of producing the same, and use of the **vaccine** for prophylaxis or **treatment** of vertebrates at risk of or suffering from disease caused by a pathogenic micro-organism. The **vaccine** is an attenuated or avirulent pathogenic micro-organism that over-expresses at least one homologous antigen encoded by at least one gene derived from the pathogenic micro-organism, and may also express a heterologous antigen.

L16 ANSWER 2 OF 47 CAPLUS COPYRIGHT 2003 ACS
AN 2003:235421 CAPLUS
DN 138:253707
TI Fusion agents contg. immunostimulating (adjuvant) and immunogenic domain as **vaccines**
IN Minion, F. Chris; Menon, Sreekumar A.; Mahairas, Gregory G.
PA Iowa State University Research Foundation, USA
SO U.S., 26 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6537552	B1	20030325	US 2000-692064	20001019
PRAI US 1999-160429P	P	19991019		

AB I fusion agents such as fusion proteins that are useful for the **treatment** and **prevention** of diseases that are susceptible to the effects of cellular (Th1 type) immune responses. Also encompassed by the invention are nucleic acids encoding the fusion proteins of the invention, vectors contg. the nucleic acids, and cells contg. the vectors. The invention includes methods of making and using the fusion agents of the invention.
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 47 MEDLINE DUPLICATE 1
AN 2003139729 MEDLINE
DN 22541561 PubMed ID: 12654848
TI Enhanced murine antigen-specific gamma interferon and immunoglobulin G2a responses by using mycobacterial **ESAT-6** sequences in DNA **vaccines**.
AU Minion F Chris; Menon Sreekumar A; Mahairas Gregory G; Wannemuehler M J
CS Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.. fcminion@iastate.edu
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 2239-43.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB The Mycobacterium **tuberculosis** protein **ESAT-6** has unusual immune stimulating activities, has been implicated in the recall of long-lived immunity, and induces protection against **tuberculosis** in mice. For many diseases caused by bacterial or viral pathogens, a strong cell-mediated immune (i.e., type 1) response is often required for recovery or protection. Therefore, it is important to design immunization regimens that induce agent-specific type 1 immunity.

We have shown in previous studies that **ESAT-6** could enhance antigen-specific type 1 immune responses in BALB/c mice against a second antigen when presented as a purified fusion protein. It was also of interest to determine if **ESAT-6** could enhance the type 1 response against a second antigen beyond that afforded by DNA **vaccination** through CpG motifs. This was tested by using gene fusions of **ESAT-6** and the Mycoplasma hyopneumoniae surface antigen P71. Modified P71 gene sequences were cloned with or without **ESAT-6** sequences into a DNA **vaccine** vector and were used to immunize mice. Splenic lymphocytes from **vaccinated** mice were tested for gamma interferon (IFN-gamma) and interleukin-10 (IL-10) secretion. Serum antibodies were examined for P71 antigen-specific isotype responses. When stimulated in vitro with purified P71 antigen, splenocytes from the **ESAT-6**:P71 **vaccinates** secreted higher levels of IFN-gamma and lower levels of IL-10 compared to those of **vaccinates** receiving the P71 construct alone. Furthermore, the immunoglobulin G2a serum antibody levels were significantly higher in the **ESAT-6**:P71 **vaccinates** compared to those of the **vaccinates** receiving P71 alone. In conclusion, **ESAT-6** was shown to enhance antigen-specific type 1 immune responses in BALB/c mice when used in DNA **vaccines**.

L16 ANSWER 4 OF 47 MEDLINE
AN 2003139677 MEDLINE
DN 22541491 PubMed ID: 12654778
TI Virulence, immunogenicity, and protective efficacy of two recombinant Mycobacterium bovis bacillus Calmette-Guerin strains expressing the antigen **ESAT-6** from Mycobacterium **tuberculosis**
.
AU Bao Lang; Chen Wei; Zhang Huidong; Wang Xiaoying
CS Research Unit of Infection and Immunity, West China Medical Center, Sichuan University, No. 17, 3rd Section, Ren Min Nan Road, Chengdu, Sichuan 610041, People's Republic of China.. baolang@wcums.edu.cn
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 1656-61.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB We constructed two recombinant Mycobacterium bovis BCG (rBCG) strains expressing **ESAT-6** of Mycobacterium **tuberculosis**, named rBCG-1 and rBCG-2. rBCG-1 contained the **ESAT-6** gene linked to BCG hsp60 and expressed a fusion protein, while rBCG-2, with a secretory sequence, could secret **ESAT-6** into the culture medium. There was no evidence for increased virulence of the two rBCG strains when we made a comparison between them and BCG with regard to organ bacterial loads, lung histology, and survival time. rBCG-1 induced significantly higher specific antibody titers and stronger cellular immune response than BCG, whereas rBCG-2 had immunogenicity similar to that of the parental BCG strain. Both rBCG-1 and rBCG-2 conferred marked protection against M. **tuberculosis** infection, yet in terms of protective efficacy, they showed no significant improvements upon conventional BCG **vaccine**.

L16 ANSWER 5 OF 47 MEDLINE
AN 2003195783 MEDLINE
DN 22554816 PubMed ID: 12667217
TI A DNA prime-live **vaccine** boost strategy in mice can augment

IFN-gamma responses to mycobacterial antigens but does not increase the protective efficacy of two attenuated strains of Mycobacterium bovis against bovine **tuberculosis**.

AU Skinner M A; Ramsay A J; Buchan G S; Keen D L; Ranasinghe C; Slobbe L; Collins D M; de Lisle G W; Buddle B M

CS AgResearch Ltd, Wallaceville Animal Research Centre, Upper Hutt, New Zealand.. margot.skinner@agresearch.co.nz

SO IMMUNOLOGY, (2003 Apr) 108 (4) 548-55.
Journal code: 0374672. ISSN: 0019-2805.

CY England: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200306

ED Entered STN: 20030429
Last Updated on STN: 20030608
Entered Medline: 20030606

AB The Mycobacterium bovis bacille Calmette-Guerin (BCG) **vaccine** has variable efficacy for both human and bovine **tuberculosis**. There is a need for improved **vaccines** or **vaccine** strategies for control of these diseases. A recently developed prime-boost strategy was investigated for **vaccination** against M. bovis infection in mice. BALB/c and C57BL/6 mice were primed with a DNA **vaccine**, expressing two mycobacterial antigens, **ESAT-6** and antigen 85 A and boosted with attenuated M. bovis strains, BCG or WAg520, a newly attenuated strain, prior to aerosol challenge. Before challenge, the antigen-specific production of interferon-gamma (IFN-gamma) was evaluated by ELISPOT and antibody responses were measured. The prime-boost stimulated an increase in the numbers of IFN-gamma producing cells compared with DNA or live **vaccination** alone, but this varied according to the attenuated **vaccine** strain, time of challenge and the strain of mouse used. Animals **vaccinated** with DNA alone generated the strongest antibody response to mycobacterial antigens, which was predominantly IgG1. BCG and WAg520 alone generally gave a 1-2 log10 reduction in bacterial load in lungs or spleen, compared to non-**vaccinated** or plasmid DNA only control groups. The prime-boost regimen was not more effective than BCG or WAg520 alone. These observations demonstrate the comparable efficacy of BCG and WAg520 in a mouse model of bovine **tuberculosis**. However, priming with the DNA **vaccine** and boosting with an attenuated M. bovis **vaccine** enhanced IFN-gamma immune responses compared to **vaccinating** with an attenuated M. bovis **vaccine** alone, but did not increase protection against a virulent M. bovis infection.

L16 ANSWER 6 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

AN 2003220512 EMBASE

TI Recent progress in the development and testing of **vaccines** against human **tuberculosis**.

AU McMurray D.N.

CS D.N. McMurray, Dept. of Med. Microbiol./Immunology, TX A/M Univ. Syst. Hlth. Sci. Center, Reynolds Medical Building, College Station, TX 77843-1114, United States. dmcmurray@tamu.edu

SO International Journal for Parasitology, (2003) 33/5-6 (547-554).
Refs: 43
ISSN: 0020-7519 CODEN: IJPYBT

CY United Kingdom

DT Journal; General Review

FS 004 Microbiology
015 Chest Diseases, Thoracic Surgery and Tuberculosis
026 Immunology, Serology and Transplantation
037 Drug Literature Index
039 Pharmacy

LA English

SL English
AB The growing pandemic of human **tuberculosis** has not been affected significantly by the widespread use of the only currently available **vaccine**, bacille Calmette Guerin. Bacille Calmette Guerin protects uniformly against serious paediatric forms of **tuberculosis** and against adult pulmonary **tuberculosis** in some parts of the world, but there are clearly populations in high-burden countries which do not benefit from the current **vaccination** regimen. New **tuberculosis vaccines** will be essential for the ultimate control of this ancient disease. Research over the past 10 years has produced literally hundreds of new **tuberculosis vaccine** candidates representing all of the major **vaccine** design strategies; protein/peptide **vaccines** in adjuvants, DNA **vaccines**, naturally and rationally attenuated strains of mycobacteria, recombinant mycobacteria and other living **vaccine** vectors expressing genes coding for immunodominant mycobacterial antigens, and non-peptide **vaccines**. Many of these **vaccines** have been tested for immunogenicity and protective efficacy in mouse and guinea pig models of low-dose pulmonary **tuberculosis**. In addition, alternative routes of **tuberculosis vaccine** delivery (e.g. oral, respiratory, gene gun) and various combinations of priming or boosting an experimental **vaccine** with bacille Calmette Guerin have been examined in relevant animal models. One of the most promising of these **vaccines** is currently in Phase I trials in human subjects, and others are expected to follow in the near future. This review will summarise the most recent progress made toward the development and preclinical evaluation of novel **vaccines** for human **tuberculosis**.

L16 ANSWER 7 OF 47 MEDLINE DUPLICATE 2
AN 2003204563 MEDLINE
DN 22610413 PubMed ID: 12692540
TI Recombinant BCG exporting **ESAT-6** confers enhanced protection against **tuberculosis**.
CM Comment in: Nat Med. 2003 May;9(5):503-4
AU Pym Alexander S; Brodin Priscille; Majlessi Laleh; Brosch Roland; Demangel Caroline; Williams Ann; Griffiths Karen E; Marchal Gilles; Leclerc Claude; Cole Stewart T
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France.
SO NATURE MEDICINE, (2003 May) 9 (5) 533-9.
Journal code: 9502015. ISSN: 1078-8956.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200306
ED Entered STN: 20030502
Last Updated on STN: 20030627
Entered Medline: 20030626
AB The live **tuberculosis vaccines** Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens **ESAT-6** (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium **tuberculosis** complex. To increase their immunogenicity and protective capacity, we complemented both **vaccines** with different constructs containing the esxA and esxB genes, which encode **ESAT-6** and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and

resulted in specific **ESAT-6**-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs **vaccinated** with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. **tuberculosis**, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L16 ANSWER 8 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2003220508 EMBASE
TI Haemolysin A and listeriolysin - Two **vaccine** delivery tools for the induction of cell-mediated immunity.
AU Dietrich G.; Viret J.-F.; Gentschev I.
CS G. Dietrich, Vaccine Research, Berna Biotech AG, Rehhagstr. 79, CH-3018, Bern, Switzerland. guido.dietrich@bernabiotech.com
SO International Journal for Parasitology, (2003) 33/5-6 (495-505).
Refs: 109
ISSN: 0020-7519 CODEN: IJPYBT
CY United Kingdom
DT Journal; General Review
FS 004 Microbiology
026 Immunology, Serology and Transplantation
036 Health Policy, Economics and Management
037 Drug Literature Index
038 Adverse Reactions Titles
LA English
SL English
AB Haemolysin A of Escherichia coli and listeriolysin of Listeria monocytogenes represent important bacterial virulence factors. While such cytolysins are usually the reason for morbidity and even mortality, **vaccine** researchers have turned haemolysin A and listeriolysin into tools for **vaccine** delivery. Both cytolysins have found widespread application in **vaccine** research and are highly suitable for the elicitation of cell-mediated immunity. In this paper, we will review **vaccine** delivery mediated by the haemolysin A secretion system and listeriolysin and will highlight their use in **vaccination** approaches against protozoan parasites.

L16 ANSWER 9 OF 47 MEDLINE
AN 2003012064 MEDLINE
DN 22406403 PubMed ID: 12518231
TI Combined recombinant DNA **vaccine** results in significant protection against Mycobacterium **tuberculosis**.
AU Pan Yi; Cai Hong; Li Shu-Xia; Tian Xia; Li Tang; Zhu Yu-Xian
CS College of Life Sciences, Peking University, Beijing 100871, China.
SO Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai), (2003 Jan) 35 (1) 71-6.
Journal code: 20730160R. ISSN: 0582-9879.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200305
ED Entered STN: 20030109
Last Updated on STN: 20030522
Entered Medline: 20030521
AB Three proteins secreted from Mycobacterium **tuberculosis**, Ag85B, **ESAT-6** and MPT63 were selected as antigens for making combined DNA **vaccine** by immunizing mice. The immune response induced by the **vaccine** and its protective efficacy were studied. It was demonstrated that when mice were immunized with the combined DNA

vaccine, the titer of antibody for Ag85B in serum increased to more than 10(5), but the titers of **ESAT-6** and MPT63 specific antibodies were undetectable. After the final immunization, the level of gamma specific for Ag85B, **ESAT-6** and MPT63 reached (17.0+/-7.0) u/ml, (6.0+/-0.8) u/ml and (11.9+/-8.0) u/ml, respectively. Mice, that were inoculated with the empty eukaryotic expression vector pJW4303 DNA, produced negligible amounts of antigen-specific INF-gamma. The combined DNA **vaccine** resulted also in significantly reduced amount of bacteria in the lungs of experimental mice. Microphotographs showed clearly that these lungs were better protected against Mycobacterium **tuberculosis** challenge than control mice. The combined DNA **vaccine** reported in this work shed new lights on the prophylactic protection against **tuberculosis**.

L16 ANSWER 10 OF 47 MEDLINE DUPLICATE 3
AN 2003165417 MEDLINE
DN 22542992 PubMed ID: 12683337
TI Up-to-date understanding of **tuberculosis** immunity.
AU Mitsuyama Masao; Akagawa Kiyoko; Kobayashi Kazuo; Sugawara Izamu; Kawakami Kazuyoshi; Yamamoto Saburo; Okada Zenshi
CS Department of Microbiology, Kyoto University Graduate School of Medicine, Yoshida-Konoecho, Sakyo-ku, Kyoto-shi, Kyoto 606-8501, Japan.. mituyama@mb.med.kyoto-u.ac.jp
SO KEKKAKU, (2003 Jan) 78 (1) 51-5. Ref: 0
Journal code: 0422132. ISSN: 0022-9776.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA Japanese
FS Priority Journals
EM 200305
ED Entered STN: 20030410
Last Updated on STN: 20030521
Entered Medline: 20030520
AB This symposium was organized to provide the up-to-date knowledge on **tuberculosis** immunity, especially on the understanding of cytokines or Th1 cells involved in pathophysiology/protective immunity and **vaccine** development. Dr. Kazuo Kobayashi (Osaka City Univ.) reported their findings on the immune response to bioactive lipid component from M. **tuberculosis**, trehalose-dimycolate (TDM) and sulfolipid (SL) in mice. Their unique and novel finding was that TDM is capable of inducing T-dependent immune response in euthymic mice. The specific immune response in TDM-immune mice was consisting of CD4+ cell response and expression of chemokines, inflammatory cytokines and then TH1-related cytokines. In contrast, SL did not show such an activity. TDM may be one of the protective antigens and may modulate the specific immune response of the host. Dr. Isamu Sugawara's group (JATA) has examined the involvement of various cytokines in the host response to aerosolic infection with virulent strain of M. **tuberculosis** by using cytokine-knockout mice. The single deletion of IFN-gamma or TNF alpha resulted in a severe lesion of multiple necrosis without granuloma, and cytokine mRNA level other than knocked out cytokine was normal, suggesting that IFN-gamma and TNF alpha are principally important cytokines. In knockout mice for IL-12 or IL-18, necrotic lesion was not induced after infection and the pathological change was not so significant as in IFN-gamma/TNF alpha knockout mice. By using IFN-gamma knockout mice, it became possible to generate a granulomatous lesion with central necrosis and cavity resembling the lesion in humans. These mouse model appeared to be useful in the analysis of pathophysiology of human **tuberculosis**. Dr. Kazuyoshi Kawakami (Ryukyu Univ.) reported the importance of TH1 cytokines in anti-tuberculous immunity. By using IL-12,

IL-18 knockout mice or double knockout mice, it was shown that IL-12 exhibits more important role than IL-18 in the protection. A possible contribution of IL-23 was also suggested. In most of the clinical cases of **tuberculosis**, the production of IL-12, IL-18 and IFN-gamma is increased, however, the group of relatively lower cytokine production did not respond well to the **treatment**. In addition, the plasma level of one of the chemokines, IP-10, was shown to be an indicator for the severity of the disease. Thus, some cytokines appear to be employable for the novel **treatment** in the near future. Dr. Saburo Yamamoto (NIH) summarized the recent advance in the understanding of biological function of CpG motifs. Immunostimulatory DNA is effective in the modulation of TH1/TH2 polarity and the enhancement of protective immunity to M. tuberulosis in animals. CpG motif (immunostimulatory DNA) appears to exert its activity by signaling cascade via TLR9 resulting in NF-kappa B activation and cytokine gene expression. Analysis of basic mechanism of action by CpG motif should pave the way to the clinical application in the future. Dr. Masaji Okada (Kinki Chuo Hospital) reported the current situation in the development of novel **vaccines** against **tuberculosis**. They have extensively constructed and examined the efficacy of various types of **vaccines** including subunit, DNA and recombinant BCG **vaccines**. Various vector systems have been tested for DNA **vaccine**. As immunizing antigens, a-Ag, **ESAT-6**, HSP65, 38kD-lipoprotein and so on have been employed. A large body of experimental data are accumulating for final evaluation, and among them, it is noteworthy to mention that HSP65DNA + IL-12DNA was 100 times more effective than conventional BCG in animal model. Among subunit **vaccines**, Mtb72f **vaccine** appears to be one of the promising candidates. In addition to the trial with various candidates, they have established a new mouse model, SCID/human PBL. This model animal has been employed for the development of **vaccine** effective for the induction of **ESAT-6**-specific human T cells.

L16 ANSWER 11 OF 47 CAPLUS COPYRIGHT 2003 ACS
AN 2002:716453 CAPLUS
DN 137:246530
TI Fusion proteins of Leishmania antigens and antigens of pathogens for diagnostic or **vaccine** use
IN Skeiky, Yasir; Brannon, Mark; Guderian, Jeffrey
PA Corixa Corporation, USA
SO PCT Int. Appl., 155 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002072792	A2	20020919	WO 2002-US8223	20020313

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI US 2001-275837P P 20010313
AB Fusion proteins of antigens of Leishmania and foreign antigens that may be useful in the diagnosis, prophylaxis or **treatment** of disease are described. The Leishmania antigen may be TSA (thiol-specific antioxidant), LeIF (initiation factor 4A), M15 or 6H. The invention also

provides an expression cassette comprising the recombinant nucleic acid mol., host cells comprising the expression cassette, compns., fusion polypeptides, and methods of their use in diagnosis or in generating a protective immune response in hosts. The genes may be codon optimized for expression in a specific host. Specifically, fusion proteins with antigens of Mycobacterium **tuberculosis** are described. Construction of codon optimized genes for fusion proteins of Leishmania antigens and Mycobacterium **tuberculosis** antigens and their expression in HEK cells is demonstrated.

L16 ANSWER 12 OF 47 MEDLINE
AN 2002271820 MEDLINE
DN 22006907 PubMed ID: 12010994
TI Correlation of **ESAT-6**-specific gamma interferon production with pathology in cattle following Mycobacterium bovis BCG **vaccination** against experimental bovine **tuberculosis**.
AU Vordermeier H Martin; Chambers Mark A; Cockle Paul J; Whelan Adam O; Simmons Jennifer; Hewinson R Glyn
CS Veterinary Laboratories Agency Weybridge, TB Research Group, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom..
mvordermeier.vla@gtnet.gov.uk
SO INFECTION AND IMMUNITY, (2002 Jun) 70 (6) 3026-32.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200206
ED Entered STN: 20020516
Last Updated on STN: 20020627
Entered Medline: 20020626
AB **Vaccine** development and the understanding of the pathology of bovine **tuberculosis** in cattle would be greatly facilitated by the definition of immunological correlates of protection and/or pathology. To address these questions, cattle were **vaccinated** with Mycobacterium bovis bacillus Calmette-Guerin (BCG) and were then challenged with virulent M. bovis. Applying a semiquantitative pathology-scoring system, we were able to demonstrate that BCG **vaccination** imparted significant protection by reducing the disease severity on average by 75%. Analysis of cellular immune responses following M. bovis challenge demonstrated that proliferative T-cell and gamma interferon (IFN-gamma) responses towards the M. bovis-specific antigen **ESAT-6**, whose gene is absent from BCG, were generally low in **vaccinated** animals but were high in all nonvaccinated calves. Importantly, the amount of **ESAT-6** -specific IFN-gamma measured by enzyme-linked immunosorbent assay after M. bovis challenge, but not the frequency of responding cells, correlated positively with the degree of pathology found 18 weeks after infection. Diagnostic reagents based on antigens not present in BCG, like **ESAT-6** and CFP-10, were still able to distinguish BCG-**vaccinated**, diseased animals from BCG-**vaccinated** animals without signs of disease. In summary, our results suggest that the determination of **ESAT-6**-specific IFN-gamma, while not a direct correlate of protection, constitutes nevertheless a useful prognostic immunological marker predicting both **vaccine** efficacy and disease severity.

L16 ANSWER 13 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2003019127 EMBASE
TI Proteome analysis of the plasma membrane of Mycobacterium **tuberculosis**.
AU Sinha S.; Arora S.; Kosalai K.; Namane A.; Pym A.S.; Cole S.T.
CS S. Sinha, Division of Biochemistry, Central Drug Research Institute, PO

Box No. 173, Lucknow 226001, India. sinhas@lycos.com
SO Comparative and Functional Genomics, (2002) 3/6 (470-483).
Refs: 51
ISSN: 1531-6912 CODEN: YESTE3
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
015 Chest Diseases, Thoracic Surgery and Tuberculosis
037 Drug Literature Index
LA English
SL English
AB The plasma membrane of Mycobacterium **tuberculosis** is likely to contain proteins that could serve as novel drug targets, diagnostic probes or even components of a **vaccine** against **tuberculosis**. With this in mind, we have undertaken proteome analysis of the membrane of M. **tuberculosis** H37Rv. Isolated membrane vesicles were extracted with either a detergent (Triton X114) or an alkaline buffer (carbonate) following two of the protocols recommended for membrane protein enrichment. Proteins were resolved by 2D-GE using immobilized pH gradient (IPG) strips, and identified by peptide mass mapping utilizing the M. **tuberculosis** genome database. The two extraction procedures yielded patterns with minimal overlap. Only two proteins, both HSPs, showed a common presence. MALDI-MS analysis of 61 spots led to the identification of 32 proteins, 17 of which were new to the M. **tuberculosis** proteome database. We classified 19 of the identified proteins as 'membrane-associated'; 14 of these were further classified as 'membrane-bound', three of which were lipoproteins. The remaining proteins included four heat-shock proteins and several enzymes involved in energy or lipid metabolism. Extraction with Triton X114 was found to be more effective than carbonate for detecting 'putative' M. **tuberculosis** membrane proteins. The protocol was also found to be suitable for comparing BCG and M. **tuberculosis** membranes, identifying **ESAT-6** as being expressed selectively in M. **tuberculosis**. While this study demonstrates for the first time some of the membrane proteins of M. **tuberculosis**, it also underscores the problems associated with proteomic analysis of a complex membrane such as that of a mycobacterium. Copyright .COPYRGT. 2002 John Wiley & Sons, Ltd.

L16 ANSWER 14 OF 47 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2002:599122 BIOSIS
DN PREV200200599122
TI Development of new **vaccines** and diagnostic reagents against **tuberculosis**.
AU Mustafa, Abu Salim (1)
CS (1) Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat, 13110: abusalim@hsc.kuniv.edu.kw Kuwait
SO Molecular Immunology, (September, 2002) Vol. 39, No. 1-2, pp. 113-119. http://www.elsevier.com/locate/molimm. print.
ISSN: 0161-5890.
DT Article
LA English
AB **Tuberculosis** (TB) is a major infectious disease problem with one-third of the world population infected, 8 million people developing the active disease and 2 million dying of TB each year. The attenuated Mycobacterium bovis Bacillus Calmette Guerin (BCG) is the only available **vaccine** against TB. However, the trials conducted in different parts of the world have shown that this **vaccine** doe not provide consistent protection against TB. The purified protein derivative (PPD) of Mycobacterium **tuberculosis** is the commonly used reagent for the diagnosis of TB. However, PPD lacks specificity because of the presence of antigens crossreactive with M. bovis BCG and other mycobacteria. The

studies to identify M. **tuberculosis** antigens and epitopes as candidates for new protective **vaccines** and specific diagnostic reagents against TB have led to the identification and characterization of several major antigens of M. **tuberculosis** including heat shock proteins (hsp) and secreted antigens present in the culture filtrate (CF) of M. **tuberculosis**. Some of these antigens have shown promise as new candidate **vaccines** (hsp60, Ag85 and **ESAT-6** , etc.) and specific diagnostic reagents (**ESAT-6** and CFP10, etc.) for TB. Moreover, in the mouse model of TB, **vaccination** with DNA-hsp60 has immunotheraputic effects and helps in eradication of persisters. In addition, identification of proper adjuvant and delivery systems has shown the promise to overcome the problem of poor immunogenicity associated with subunit and peptide based **vaccines**. More recently, the comparison of the genome sequence of M. **tuberculosis** with M. bovis BCG and other mycobacteria has led to the identification of M. **tuberculosis**-specific genomic regions. Evaluation of these regions for encoding proteins with immunological reactivity can lead to the identification of additional antigens of M. **tuberculosis** useful as new **vaccines** and reagents for specific diagnosis of TB.

L16 ANSWER 15 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2002421778 EMBASE
TI A novel TB **vaccine**; towards a strategy based on our understanding of BCG failure.
AU Agger E.M.; Andersen P.
CS E.M. Agger, Dept. of Infectious Disease Immunol., Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark. pa@ssi.dk
SO Vaccine, (22 Nov 2002) 21/1-2 (7-14).
Refs: 79
ISSN: 0264-410X CODEN: VACCDE
PUI S 0264-410X(02)00447-4
CY United Kingdom
DT Journal; General Review
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB The protection afforded by the currently available **tuberculosis** **vaccine**, bacillus Calmette-Guerin (BCG) is insufficient and new **vaccine** strategies are urgently needed. Progress in our understanding of the immunological deficits of BCG combined with novel knowledge on genetics of mycobacteria has paved the way for promising new **vaccine** strategies. These include recombinant modified BCG **vaccines**, attenuated strains of Mycobacterium **tuberculosis** , and various non-live candidates such as DNA and subunit **vaccines** . Decisive for transforming technical progress into a novel **tuberculosis** (TB) **vaccine** strategy is the recent advance in our understanding of the failure of BCG in the third world and the interaction between this **vaccine** and environmental mycobacteria.

L16 ANSWER 16 OF 47 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 5
AN 2002-147798 [19] WPIDS
DNC C2002-045868
TI Composition comprising MTB39 antigen and MTB32A antigen from Mycobacterium species, useful for eliciting immune response in a subject.
DC B04 D16
IN ALDERSON, M; REED, S; SKEIKY, Y
PA (CORI-N) CORIXA CORP
CYC 95
PI WO 2001098460 A2 20011227 (200219)* EN 136p

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU
SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001068678 A 20020102 (200230)
ADT WO 2001098460 A2 WO 2001-US19959 20010620; AU 2001068678 A AU 2001-68678 20010620
FDT AU 2001068678 A Based on WO 200198460
PRAI US 2001-265737P 20010201; US 2000-597796 20000620
AB WO 200198460 A UPAB: 20020321
NOVELTY - A composition (I) comprising a MTB39 antigen (A1) (comprising a sequence of 263 or 391 amino acids fully defined in the specification) and a MTB32A antigen (A2) (comprising a sequence of 355 or 330 amino acids fully defined in the specification), or their immunogenic fragments, from a Mycobacterium sp. of the **tuberculosis** complex, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an expression cassette (II) comprising a nucleic acid encoding A1 and a nucleic acid encoding A2;

(2) an isolated nucleic acid (III) encoding (II), where at least one amino acid in the active site triad of the MTB32A antigen has been substituted by a different amino acid;

(3) an isolated nucleic acid (IIIa) encoding a fusion polypeptide comprising (III);

(4) an isolated MTB32A polypeptide (IV) from a Mycobacterium sp. of the **tuberculosis** complex, has at least one amino acid in the active site triad of the MTB32A antigen substituted by a different amino acid;

(5) a fusion polypeptide (FP1) comprising (IV);

(6) an isolated nucleic acid (V) encoding a fusion polypeptide comprising A1 and an antigen comprising at least 195 amino acids from the N-terminus of (IV);

(7) a nucleic acid encoding a fusion polypeptide comprising (V);

(8) an isolated polypeptide (VI) encoding a fusion polypeptide comprising A1 and an antigen comprising at least 195 amino acids from (IV);

(9) a fusion polypeptide (FP2) comprising (Va); and

(10) a composition (C) comprising (III), (IV), (V) or (VI).

ACTIVITY - Tuberculostatic; immunostimulant.

MECHANISM OF ACTION - **Vaccine**.

Guinea pigs were immunized with adjuvants (SBAS1, SBAS2 or ASAS7 plus A1(OH)3), MTB72F fusion protein in adjuvant, or TbH9 plus Ra35 antigen composition at a dosage of 4 micro g each of TbH9 and Ra35, and 8 micro g of MTB72F. Second immunization was carried out after 3 weeks and third immunization approximately after two and a half weeks. 10 micro g of antigen was used as a prechallenge to determine antigenicity and delayed type hypersensitivity (DTH). Weight loss and death of the animals were monitored. The results for DTH were positive to the immunizing antigens. Reactions to individual antigens or the fusion protein were comparable. Guinea pigs **vaccinated** with MTB72F fusion protein afforded protection compared to those immunized with a mixture of antigens.

USE - (I) and (II) are useful for eliciting an immune response in a mammal, e.g., human, immunized with BCG (claimed). (I) and (II) are useful in diagnosis, **treatment** and **prevention** of Mycobacterium infection. (I), the fusion proteins and the polynucleotides are useful as diagnostic tools in patients infected with Mycobacterium, in vitro and in vivo assays for detecting humoral antibodies or cell-mediated immunity against M. **tuberculosis** for diagnosis of an infection or monitoring of disease progression, as immunogens to generate or elicit a protective immune response in a patient and for raising anti-M. **tuberculosis** antibodies in a non-human animal. (IV) is useful as

in vivo diagnostic agent for intradermal skin test.

ADVANTAGE - Compositions and fusion proteins/polynucleotides that contain at least two heterologous M. **tuberculosis** coding sequences or antigens are highly antigenic and upon administration to a patient increase the sensitivity of **tuberculosis** sera.

Monkeys immunized with a composition comprising a mixture of two antigens (MTB72F and MTB8.4) showed weight stabilization and low erythrocyte sedimentation rate (ESR) (max 10) compared to those immunized with single antigen (MTB8.4) which showed weight loss and high ESR (max 30).

Dwg.0/7

L16 ANSWER 17 OF 47 CAPLUS COPYRIGHT 2003 ACS
AN 2001:265269 CAPLUS
DN 134:309685
TI Fusion proteins of Mycobacterium **tuberculosis**
IN Skeiky, Yasir; Reed, Steven; Houghton, Raymond L.; Mcneill, Patricia D.; Dillon, Davin C.; Lodes, Michael L.
PA Corixa Corporation, USA
SO PCT Int. Appl., 168 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001024820	A1	20010412	WO 2000-US28095	20001010
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1229931	A1	20020814	EP 2000-970785	20001010
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
PRAI US 1999-158338P	P	19991007		
US 1999-158425P	P	19991007		
WO 2000-US28095	W	20001010		

AB The present invention relates to fusion proteins contg. at least two Mycobacterium species antigens. In particular, it relates to nucleic acids encoding fusion proteins that include two or more individual M. **tuberculosis** antigens, which increase serol. sensitivity of sera from individuals infected with **tuberculosis**, and methods for their use in the diagnosis, **treatment**, and **prevention** of **tuberculosis** infection.

RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 18 OF 47 MEDLINE DUPLICATE 6
AN 2001567381 MEDLINE
DN 21528960 PubMed ID: 11673535
TI Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium **tuberculosis**-infected individuals: associations with clinical disease state and effect of **treatment**.
AU Pathan A A; Wilkinson K A; Klenerman P; McShane H; Davidson R N; Pasvol G; Hill A V; Lalvani A
CS Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.

combined prime-boost **vaccination** did not considerably enhance protection.

L16 ANSWER 20 OF 47 MEDLINE DUPLICATE 8
AN 2001248071 MEDLINE
DN 21189184 PubMed ID: 11292688
TI Protection of mice with a **tuberculosis** subunit **vaccine** based on a fusion protein of antigen 85b and **esat-6**.
AU Weinrich Olsen A; van Pinxteren L A; Meng Okkels L; Birk Rasmussen P; Andersen P
CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (2001 May) 69 (5) 2773-8.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200105
ED Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510
AB In this study, we investigated the potential of a **tuberculosis** subunit **vaccine** based on fusion proteins of the immunodominant antigens **ESAT-6** and antigen 85B. When the fusion proteins were administered to mice in the adjuvant combination dimethyl dioctadecylammonium bromide-monophosphoryl lipid A, a strong dose-dependent immune response was induced to both single components as well as to the fusion proteins. The immune response induced was accompanied by high levels of protective immunity and reached the level of Mycobacterium bovis BCG-induced protection over a broad dose range. The **vaccine** induced efficient immunological memory, which remained stable 30 weeks postvaccination.

L16 ANSWER 21 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001113274 EMBASE
TI **Tuberculosis** subunit **vaccine** development: On the role of interferon-.gamma..
AU Agger E.M.; Andersen P.
CS P. Andersen, Department of TB Immunology, Statens Serum Institute, Artillerivej 5, 2300 Copenhagen S, Denmark. pa@ssi.dk
SO Vaccine, (21 Mar 2001) 19/17-19 (2298-2302).
Refs: 36
ISSN: 0264-410X CODEN: VACCDE
PUI S 0264-410X(00)00519-3
CY United Kingdom
DT Journal; Conference Article
FS 015 Chest Diseases, Thoracic Surgery and Tuberculosis
017 Public Health, Social Medicine and Epidemiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB **Tuberculosis** (TB) remains a major global health problem and subunit **vaccines** for the control of the disease are presently under development. This **vaccine** strategy requires an in vitro correlate of protection for the identification of relevant **vaccine** candidate antigens and for monitoring the induction of a protective cell-mediated immune response after **vaccination**. New studies of experimental **vaccines** in the mouse model of TB support interferon-.gamma. as a relevant marker for the induction of a protective immune response. In contrast, searching for immunodominant antigens capable of inducing strong interferon-.gamma. responses in PPD positive healthy or TB infected individuals may not identify all relevant candidate

antigens for inclusion in a novel TB subunit **vaccine**. .COPYRGT. 2001 Elsevier Science Ltd.

L16 ANSWER 22 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001231117 EMBASE
TI Enhanced contact tracing and spatial tracking of Mycobacterium **tuberculosis** infection by enumeration of antigen-specific T cells.
AU Lalvani A.; Pathan A.A.; Durkan H.; Wilkinson K.A.; Whelan A.; Deeks J.J.; Reece W.H.H.; Latif M.; Pasvol G.; Hill A.V.S.
CS Dr. A. Lalvani, Nuffield Dept. of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom. ajit.lalvani@ndm.ox.ac.uk
SO Lancet, (23 Jun 2001) 357/9273 (2017-2021). Refs: 30 ISSN: 0140-6736 CODEN: LANCAO
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
006 Internal Medicine
037 Drug Literature Index
LA English
SL English
AB Background: Identification of individuals latently infected with Mycobacterium **tuberculosis** is an important part of **tuberculosis** control. The current method, the tuberculin skin test (TST), has poor specificity because of the antigenic cross-reactivity of purified protein derivative (PPD) with M bovis BCG **vaccine** and environmental mycobacteria. **ESAT-6** is a secreted antigen that is highly specific for M **tuberculosis** complex, but is absent from M bovis BCG. With an enzyme-linked immunospot (ELISPOT) assay for interferon gamma, we have identified **ESAT-6** -specific T cells as an accurate marker of M **tuberculosis** infection. Methods: We did a prospective, masked study of 50 healthy contacts, with varying but well defined degrees of exposure to M **tuberculosis**, who attended an urban contact-tracing clinic. We assessed and compared the efficacy of our assay and TST for detection of symptomless infected individuals by correlation of test results with the degree of exposure to an infectious index case. Findings: The **ESAT** -**6** ELISPOT assay results had a strong positive relation with increasing intensity of exposure (odds ratio=9.0 per unit increase in level of exposure [95% CI 2.6-31.6], p=0.001), whereas TST results had a weaker relation with exposure (1.9 [1.0-3.5], p=0.05). By contrast, ELISPOT results were not correlated with BCG **vaccination** status (p=0.7), whereas TST results were significantly more likely to be positive in BCG-**vaccinated** contacts (12.1 [1.3-115.7], p=0.03). Interpretation: This new antigen-specific T cell-based assay could allow more accurate identification of symptom-free individuals recently exposed to M **tuberculosis**, and thereby help to improve **tuberculosis** control.

L16 ANSWER 23 OF 47 MEDLINE
AN 2001638720 MEDLINE
DN 21546626 PubMed ID: 11687445
TI Tuberculin skin testing compared with T-cell responses to Mycobacterium **tuberculosis**-specific and nonspecific antigens for detection of latent infection in persons with recent **tuberculosis** contact.
AU Arend S M; Engelhard A C; Groot G; de Boer K; Andersen P; Ottenhoff T H; van Dissel J T
CS Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands.. s.m.arend@lumc.nl
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2001 Nov) 8 (6) 1089-96. Journal code: 9421292. ISSN: 1071-412X.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200201
ED Entered STN: 20011107
Last Updated on STN: 20020128
Entered Medline: 20020125
AB The tuberculin skin test (TST) is used for the identification of latent **tuberculosis** (TB) infection (LTBI) but lacks specificity in Mycobacterium bovis BCG-**vaccinated** individuals, who constitute an increasing proportion of TB patients and their contacts from regions where TB is endemic. In previous studies, T-cell responses to **ESAT-6** and CFP-10, M. **tuberculosis**-specific antigens that are absent from BCG, were sensitive and specific for detection of active TB. We studied 44 close contacts of a patient with smear-positive pulmonary TB and compared the standard screening procedure for LTBI by TST or chest radiographs with T-cell responses to M. **tuberculosis**-specific and nonspecific antigens. Peripheral blood mononuclear cells were cocultured with **ESAT-6**, CFP-10, TB10.4 (each as recombinant antigen and as a mixture of overlapping synthetic peptides), M. **tuberculosis** sonicate, purified protein derivative (PPD), and short-term culture filtrate, using gamma interferon production as the response measure. LTBI screening was by TST in 36 participants and by chest radiographs in 8 persons. Nineteen contacts were categorized as TST negative, 12 were categorized as TST positive, and 5 had indeterminate TST results. Recombinant antigens and peptide mixtures gave similar results. Responses to TB10.4 were neither sensitive nor specific for LTBI. T-cell responses to **ESAT-6** and CFP-10 were less sensitive for detection of LTBI than those to PPD (67 versus 100%) but considerably more specific (100 versus 72%). The specificity of the TST or in vitro responses to PPD will be even less when the proportion of BCG-**vaccinated** persons among TB contacts evaluated for LTBI increases.

L16 ANSWER 24 OF 47 MEDLINE DUPLICATE 9
AN 2002024114 MEDLINE
DN 21360002 PubMed ID: 11467375
TI Uncommon presentations of **tuberculosis**: the potential value of a novel diagnostic assay based on the Mycobacterium **tuberculosis** -specific antigens **ESAT-6** and CFP-10.
AU Arend S M; Ottenhoff T H; Andersen P; van Dissel J T
CS Department of Infectious Diseases, Leiden University Medical Center, The Netherlands.. s.m.arend@lumc.nl
SO INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE, (2001 Jul) 5 (7) 680-6.
Journal code: 9706389. ISSN: 1027-3719.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011205
AB SETTING: Leiden University Medical Center, Leiden, the Netherlands. OBJECTIVE: To illustrate the potential value of a recently developed diagnostic assay for detection of **tuberculosis** (TB), based on T cell responses to the early secreted antigenic target 6 kDa protein (**ESAT-6**) and culture filtrate protein 10 (CFP-10). These antigens are Mycobacterium **tuberculosis** specific because they are expressed by M. **tuberculosis** but absent from M. bovis bacille Calmette-Guerin (BCG) and most environmental mycobacteria. In recent studies, the assay had a high sensitivity and specificity for

derivatives (PPDs), which are poorly-defined mixtures containing many individual antigenic components. It is known that false-positive responses to these reagents can occur in cattle which are not infected with TB, largely because of that antigenic complexity. This paper reviews recent approaches to the characterization of more precisely defined diagnostic tools which can be used to develop tests with greater specificity. For example, the low mass secreted protein **ESAT-6** has been shown to be capable of differentiating TB-infected cattle from those which develop responsiveness to PPD through contact with environmental mycobacteria or **vaccination** with BCG. The information which has accumulated in recent years has shown that the increased specificity is associated with some decrease in test sensitivity, but the overall advantages of being able to make precise diagnostic decisions will have significant advantages in many situations.

L16 ANSWER 28 OF 47 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-303438 [26] WPIDS
DNC C2000-092041
TI New ligand presenting assemblies useful for diagnosis, **treatment** and **prevention** of diseases caused by e.g. viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer-related compounds, cell adhesion molecules.
DC B04 D16
IN HOLM, A; JORGENSEN, R M; OSTERGAARD, S; THEISEN, M
PA (HOLM-I) HOLM A; (STAT-N) STATENS SERUMINSTITUT; (STAT-N) STATENS SERUM INST
CYC 90
PI WO 2000018791 A1 20000406 (200026)* EN 79p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9960783 A 20000417 (200035)
EP 1117677 A1 20010725 (200143) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK RO SI
ADT WO 2000018791 A1 WO 1999-DK510 19990929; AU 9960783 A AU 1999-60783 19990929; EP 1117677 A1 EP 1999-947256 19990929, WO 1999-DK510 19990929
FDT AU 9960783 A Based on WO 200018791; EP 1117677 A1 Based on WO 200018791
PRAI DK 1998-1233 19980929
AB WO 200018791 A UPAB: 20000531
NOVELTY - A method for preparing ligand presenting assemblies (LPAs) using solid phase synthesis, ring-formation with a di-, tri- or tetracarboxylic acid and cleavage, is new.

DETAILED DESCRIPTION - The method for preparing a ligand presenting assembly (LPA) enabling presentation of desired sequences comprises:

(a) providing by solid phase synthesis, or fragment coupling, ligands comprising desired sequences, the ligands being attached to a solid phase;

(b) if necessary, deprotecting any N-terminal amino groups while the ligands are still attached to the solid phase;

(c) reacting the ligands having unprotected N-terminal amino groups with an achiral di-, tri- or tetracarboxylic acid, to provide a construct having a ring structure; and

(d) cleaving the construct from the solid phase, to provide an LPA comprising ligands having free C-terminal groups.

INDEPENDENT CLAIMS are also included for the following:

(1) an LPA obtained by the novel method;

(2) an immunological composition for raising an immune response in an animal, including a human, comprising the LPA of (1);

(3) a method for generating antibodies in an animal, including a human, comprising administering an antibody-generating amount of the LPA

of (1); and

(4) a kit for use in the diagnosis of infections caused by viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer related compounds, cell adhesion molecules, neurotropic factors, fungi, parasites, or Borrelia burgdorferi sensu lato, comprises an LPA of (1) together with a device for detecting or visualizing binding between the LPA and the substance to be detected.

ACTIVITY - Antiviral; antibacterial; antiallergic; immunosuppressive; immunostimulant; cytostatic; antifungal; antiparasitic; neuroprotective.

MECHANISM OF ACTION - **Vaccine**. The capability of an LPA to induce a humoral immune response was studied by immunizing mice with LPA-VI (derived from the antigenic sequence of OspC from BB ProValValAlaGluSerProLysLysPro). 5 female C57 black mice (age 6-8 weeks) were immunized twice, intraperitoneally, with a 2-week interval and were bled on days 0, 14, and 28. Each immunization dose contained 5 mu g of LPA-VI dissolved in 0.25ml 0.9% NaCl and supplemented with 1mg Al(OH)3 and 0.25ml Freunds incomplete adjuvant. The serum samples were diluted 200-fold and then tested by Enzyme linked immunosorbant assay (ELISA) for Ig reactivity against OspC produced in recombinant form (see WO97/42221) and LPA-I. It was concluded that LPA-VI is highly effective in inducing antibodies against the C-terminal B-cell epitope of OspC since the mice generated antibodies which recognize both recombinant OspC and LPA-I.

USE - The LPAs can be used for raising an immune response in an animal (claimed). They can be used in **vaccines** and for generating antibodies in an animal (claimed). They can be used for the **treatment**, alleviation or prophylaxis of diseases caused by viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer related compounds, cell adhesion molecules, neurotropic factors, fungi or parasites (claimed). They can also be used for detection and diagnosis.

ADVANTAGE - Using the method it is possible to prepare very long ring systems interconnected by reaction with the achiral di-, tri- or tetracarboxylic acid. The ring structure formed between desired sequences further enables additional presentation of desired sequences and chemical moieties. The LPAs provide very flexible systems for polyfunctional constructs, and furthermore, products of high purity are obtained.
Dwg.0/12

L16 ANSWER 29 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001185855 EMBASE
TI Human CD8(+) T cells specific for Mycobacterium **tuberculosis** secreted antigens in **tuberculosis** patients and healthy BCG-**vaccinated** controls in the Gambia.
AU Smith S.M.; Klein M.R.; Malin A.S.; Sillah J.; Huygen K.; Andersen P.; McAdam K.P.W.J.; Dockrell H.M.
CS S.M. Smith, Immunology Unit, London Sch. of Hyg. and Trop. Med., Keppel Street, London WC1E 7HT, United Kingdom. steven.smith@lshtm.ac.uk
SO Infection and Immunity, (2000) 68/12 (7144-7148).
Refs: 26
ISSN: 0019-9567 CODEN: INFIBR
CY United States
DT Journal; Article
FS 004 Microbiology
037 Drug Literature Index
LA English
SL English
AB Intracellular flow cytometry analysis of perforin production by CD8(+) T cells showed levels were greatly reduced in **tuberculosis** (TB) patients compared to healthy controls. Reduced cytotoxic-T-lymphocyte activity was also obtained with CD8(+) T cells from TB patients compared to healthy controls in The Gambia. A change in antigen recognition was noted between the two groups of donors: in addition to recognition of Ag85A and Ag85B, as seen in healthy donors, a prominent **ESAT-**

Journal code: 9203213. ISSN: 1058-4838.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200009
ED Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000906
AB A scientific review of the recent sharp increase in bovine **tuberculosis** in Great Britain has concluded that the development of a cattle **vaccine** holds the best prospect for long-term disease control. It is important to develop a diagnostic test that differentiates between **vaccinated** and Mycobacterium bovis-infected animals, to ensure that test-and-slaughter control strategies can continue alongside **vaccination**. The mycobacterial antigens **ESAT-6**, MPB64, and MPB83 are expressed at high levels in M. bovis but are expressed at low levels or not at all in bacille Calmette-Guerin (BCG) Pasteur. Promiscuous bovine T cell epitopes of these antigens were identified and formulated into a peptide cocktail. This cocktail and a cocktail composed of recombinant forms of the 3 antigens was able to distinguish cattle infected with virulent M. bovis from those **vaccinated** with BCG and from those sensitized to avian tuberculin in lymphocyte transformation and interferon-gamma assays.

L16 ANSWER 34 OF 47 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:2713 BIOSIS
DN PREV200100002713
TI Immunogenicity of DNA **vaccines** encoding **tuberculosis** early secretory protein **ESAT-6** fused to chemokines.
AU Azzazy, H. M. E. (1); Izumikawa, T. (1); Cummings, P. (1); Zimmerman, D. H.
CS (1) Univ. of Maryland Sch. of Med., Baltimore, MD USA
SO Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 252. print.
Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy Toronto, Ontario, Canada September 17-20, 2000
DT Conference
LA English
SL English

L16 ANSWER 35 OF 47 MEDLINE DUPLICATE 13
AN 2000072687 MEDLINE
DN 20072687 PubMed ID: 10603390
TI Comparative evaluation of low-molecular-mass protéins from Mycobacterium **tuberculosis** identifies members of the **ESAT-6** family as immunodominant T-cell antigens.
AU Skjot R L; Oettinger T; Rosenkrands I; Ravn P; Brock I; Jacobsen S; Andersen P
CS Department of TB Immunology, Statens Serum Institut, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (2000 Jan) 68 (1) 214-20.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
ED Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000111
AB Culture filtrate from Mycobacterium **tuberculosis** contains protective antigens of relevance for the generation of a new

antituberculosis **vaccine**. We have identified two previously uncharacterized M. **tuberculosis** proteins (TB7.3 and TB10.4) from the highly active low-mass fraction of culture filtrate. The molecules were characterized, mapped in a two-dimensional electrophoresis reference map of short-term culture filtrate, and compared with another recently identified low-mass protein, CFP10 (F. X. Berthet, P. B. Rasmussen, I. Rosenkrands, P. Andersen, and B. Gicquel. Microbiology 144:3195-3203, 1998), and the well-described **ESAT-6** antigen. Genetic analyses demonstrated that TB10.4 as well as CFP10 belongs to the **ESAT-6** family of low-mass proteins, whereas TB7.3 is a low-molecular-mass protein outside this family. The proteins were expressed in Escherichia coli, and their immunogenicity was tested in cultures of peripheral blood mononuclear cells from human **tuberculosis** (TB) patients, Mycobacterium bovis BCG-**vaccinated** donors, and nonvaccinated donors. The two **ESAT-6** family members, TB10.4 and CFP10, were very strongly recognized and induced gamma interferon release at the same level (CFP10) as or at an even higher level (TB10.4) than **ESAT-6**. The non-**ESAT-6** family member, TB7.3, for comparison, was recognized at a much lower level. CFP10 was found to distinguish TB patients from BCG-**vaccinated** donors and is, together with **ESAT-6**, an interesting candidate for the diagnosis of TB. The striking immunodominance of antigens within the **ESAT-6** family is discussed, and hypotheses are presented to explain this targeting of the immune response during TB infection.

L16 ANSWER 36 OF 47 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1999-04677 BIOTECHDS
TI New nucleic acid containing regulator and LHP gene of Mycobacterium **tuberculosis**;
used as immunogens and **vaccines** in Mycobacterium **tuberculosis** infection diagnosis and **prevention**
AU Gicquel B; Berthet F X; Andersen P; Rasmussen P B
PA Inst.Pasteur-Paris; Statens-Serum-Inst.Copenhagen
LO Paris, France; Copenhagen, Denmark.
PI WO 9904005 28 Jan 1999
AI WO 1998-IB1091 16 Jul 1998
PRAI US 1997-52631 16 Jul 1997
DT Patent
LA English
OS WPI: 1999-132249 [11]
AB A nucleic acid (A) with a sequence of approximately 1,300 bp, or fragments consisting of bases 1-524, 1-481 or 525-826 of that sequence, or their biologically active derivatives, are claimed. Alternatively (A) contains at least 12 consecutive nucleotides of the sequence, is the complement of one of the fragments, or hybridizes under stringent conditions to one of the fragments. Also claimed are nucleic acids (B) containing the fragments of (A) fused to a sequence encoding another protein, recombinant vectors containing (A) or (B), and host cells transformed by those vectors. The claims also cover proteins produced by those cells, and their oligomers and antigenic fragments. Also covered are polyclonal or monoclonal antibodies specific for the proteins or oligomers, and DNA probes and DNA primers derived from (A), with given DNA sequences. The proteins are for use as immunogens and **vaccines** to protect against Mycobacterium **tuberculosis** complex bacteria, and for diagnosis of M. **tuberculosis** infection. The products can also be used to detect Mycobacterium bovis. The proteins encoded by (A) induce an increased protective immune response. (88pp)

L16 ANSWER 37 OF 47 MEDLINE DUPLICATE 14
AN 1999386877 MEDLINE
DN 99386877 PubMed ID: 10456931

TI Immunogenicity of DNA **vaccines** expressing **tuberculosis** proteins fused to tissue plasminogen activator signal sequences.
AU Li Z; Howard A; Kelley C; Delogu G; Collins F; Morris S
CS Laboratory of Mycobacteria, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892, USA.
SO INFECTION AND IMMUNITY, (1999 Sep) 67 (9) 4780-6.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
ED Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991005
AB Novel **tuberculosis** DNA **vaccines** encoding native **ESAT-6**, MPT-64, KatG, or HBHA mycobacterial proteins or the same proteins fused to tissue plasminogen activator (TPA) signal sequences were evaluated for their capacity to elicit humoral, cell-mediated, and protective immune responses in **vaccinated** mice. While all eight plasmids induced specific humoral responses, the constructs expressing the TPA fusions generally evoked higher antibody responses in **vaccinated** hosts. Although most of the DNA **vaccines** tested induced a substantial gamma interferon response in the spleen, the antigen-specific lung responses were 2- to 10-fold lower than the splenic responses at the time of challenge. DNA **vaccines** encoding the **ESAT-6**, MPT-64, and KatG antigens fused to TPA signal sequences evoked significant protective responses in mice aerogenically challenged with low doses of Mycobacterium **tuberculosis** Erdman 17 to 21 days after the final immunization. However, the protective response induced by live Mycobacterium bovis BCG **vaccine** was greater than the response induced by any of the DNA **vaccines** tested. These results suggest that the **tuberculosis** DNA **vaccines** were able to elicit substantial immune responses in suitably **vaccinated** mice, but further refinements to the constructs or the use of alternative immunization strategies will be needed to improve the efficacy of these **vaccine** candidates.

L16 ANSWER 38 OF 47 MEDLINE
AN 1999184991 MEDLINE
DN 99184991 PubMed ID: 10085007
TI Differential protective efficacy of DNA **vaccines** expressing secreted proteins of Mycobacterium **tuberculosis**.
AU Kamath A T; Feng C G; Macdonald M; Briscoe H; Britton W J
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, New South Wales 2042, Australia.
SO INFECTION AND IMMUNITY, (1999 Apr) 67 (4) 1702-7.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990511
Last Updated on STN: 20030111
Entered Medline: 19990426
AB The development of more-effective antituberculosis **vaccines** would assist in the control of the global problem of infection with Mycobacterium **tuberculosis**. One recently devised **vaccination** strategy is immunization with DNA plasmids encoding individual microbial genes. Using the genes for the M. **tuberculosis** secreted proteins MPT64 (23 kDa), Ag85B (30 kDa), and

respond. In summary, our results suggest that peptide and protein cocktails can be designed to discriminate between M. bovis infection and BCG **vaccination**.

L16 ANSWER 40 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2000011618 EMBASE
TI Human T cell responses to the **ESAT-6** antigen from Mycobacterium **tuberculosis**.
AU Ravn P.; Demissie A.; Eguale T.; Wondwosson H.; Lein D.; Amoudy H.A.; Mustafa A.S.; Jensen A.K.; Holm A.; Rosenkrands I.; Oftung F.; Olobo J.; Von Reyn F.; Andersen P.
CS Dr. P. Andersen, Dept. of TB Immunology, Statens Serum Institut, Artillerivej 5, 2300 S, Copenhagen, Denmark. pa@ssi.dk
SO Journal of Infectious Diseases, (1999) 179/3 (637-645).
Refs: 47
ISSN: 0022-1899 CODEN: JIDIAQ
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Human T cell responses to **ESAT-6** and eight synthetic overlapping peptides were investigated in **tuberculosis** (TB) patients and control subjects from regions of high and low endemicity for TB. **ESAT-6** was recognized by 65% of all tuberculin purified protein derivative-responsive TB patients, whereas only 2 of 29 bacille Calmette-Guerin-**vaccinated** Danish healthy donors recognized this molecule. In Ethiopia, a high frequency (58%) of healthy contacts of TB patients recognized **ESAT-6**. All of the peptides were recognized by some donors, indicating that the molecule holds multiple epitopes. Danish and Ethiopian patients differed in the fine specificity of their peptide responses. Recognition of the C-terminal region (aa 72-95) was predominant in Danish patients, whereas recognition of aa 42-75 was predominant in Ethiopia. The relationship of these differences to the distribution of HLA types in the two populations is discussed. This study demonstrates that **ESAT-6** is frequently recognized during early infection and holds potential as a component of a future TB-specific diagnostic reagent.

L16 ANSWER 41 OF 47 MEDLINE
AN 1999091871 MEDLINE
DN 99091871 PubMed ID: 9874655
TI Differentiation between Mycobacterium bovis BCG-**vaccinated** and M. bovis-infected cattle by using recombinant mycobacterial antigens.
AU Buddle B M; Parlane N A; Keen D L; Aldwell F E; Pollock J M; Lightbody K; Andersen P
CS AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand.. buddleb@agresearch.cri.nz
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1999 Jan) 6 (1) 1-5.
Journal code: 9421292. ISSN: 1071-412X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199903
ED Entered STN: 19990316
Last Updated on STN: 19990316
Entered Medline: 19990301
AB **Tuberculosis** continues to be a worldwide problem for both humans and animals. The development of tests to differentiate between infection with Mycobacterium **tuberculosis** or Mycobacterium bovis and

vaccination with M. bovis BCG could greatly assist in the diagnosis of early infection as well as enhance the use of **tuberculosis vaccines** on a wider scale. Recombinant forms of four major secreted proteins of M. bovis-MPB59, MPB64, MPB70, and **ESAT-6**-were tested in a whole-blood gamma interferon (IFN-gamma) assay for differentiation between cattle **vaccinated** with BCG and those experimentally infected with M. bovis. BCG **vaccination** induced minimal protection in the present study, with similar numbers of animals infected with M. bovis in BCG-**vaccinated** and nonvaccinated groups. Following **vaccination** with BCG, the animals produced moderate IFN-gamma responses to bovine purified protein derivative (PPDB) but very weak responses to the recombinant antigens. Cattle from both the BCG-**vaccinated** and nonvaccinated groups which were M. bovis culture positive following challenge produced IFN-gamma responses to PPDB and **ESAT-6** which were significantly stronger than those observed in the corresponding M. bovis culture-negative animals. IFN-gamma responses to MPB59, MPB64, and MPB70 were significantly weaker, and these antigens could not discriminate between **vaccinated** animals which develop disease and the culture-negative animals. The results of the study indicate that of the four antigens tested in the IFN-gamma assay, only **ESAT-6** would be suitable for differentiating BCG-**vaccinated** animals from those infected with bovine **tuberculosis**.

L16 ANSWER 42 OF 47 WPIDS (C) 2003 THOMSON DERWENT
AN 1998-542705 [46] WPIDS
CR 1999-347282 [29]
DNN N1998-422423 DNC C1998-163143
TI New isolated mycobacteria polypeptides and nucleic acids - used for developing products for the diagnosis of or **vaccination** against mycobacterial infections, particularly **tuberculosis**.
DC B04 D16 S03
IN ANDERSEN, P; FLORIO, W; NIELSEN, R; OETTINGER, T; RASMUSSEN, P B; ROSENKRANDS, I; WELDINGH, K; SKJOT, R; OLSEN, A W; SKJOT, R L V
PA (STAT-N) STATENS SERUM INST; (STAT-N) STATENS SERUMINSTITUT; (ANDE-I) ANDERSEN P; (OLSE-I) OLSEN A W; (RASM-I) RASMUSSEN P B; (SKJO-I) SKJOT R L V
CYC 82
PI WO 9844119 A1 19981008 (199846)* EN 163p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9868204 A 19981022 (199910)
EP 972045 A1 20000119 (200009) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
NZ 504951 A 20010629 (200140)
JP 2001515359 W 20010918 (200169) 280p
AU 740545 B 20011108 (200176)
US 2002094336 A1 20020718 (200254)
US 2002176867 A1 20021128 (200281)
ADT WO 9844119 A1 WO 1998-DK132 19980401; AU 9868204 A AU 1998-68204 19980401; EP 972045 A1 EP 1998-913536 19980401, WO 1998-DK132 19980401; NZ 504951 A NZ 1998-504951 19981008, WO 1998-DK438 19981008; JP 2001515359 W JP 1998-541074 19980401, WO 1998-DK132 19980401; AU 740545 B AU 1998-68204 19980401; US 2002094336 A1 Provisional US 1997-44624P 19970418, Provisional US 1998-70488P 19980105, Div ex US 1998-50739 19980330, US 2001-791171 20010220; US 2002176867 A1 Provisional US 1997-44624P 19970418, Provisional US 1998-70488P 19980105, CIP of US 1998-246191 19981230, US 2001-805427 20010313

FDT AU 9868204 A Based on WO 9844119; EP 972045 A1 Based on WO 9844119; NZ 504951 A Based on WO 9924577; JP 2001515359 W Based on WO 9844119; AU 740545 B Previous Publ. AU 9868204, Based on WO 9844119

PRAI US 1998-70488P 19980105; DK 1997-376 19970402; US 1997-44624P 19970418; DK 1997-1277 19971110

AB WO 9844119 A UPAB: 20021216

A pure polypeptide fragment (I) is new, which comprises: (a) an amino acid sequence selected from one of the sequences shown; (b) a subsequence of (I) which has a length of at least 6 amino acid residues, being immunologically equivalent to (I) with respect to the ability of evoking a protective immune response against infections with mycobacteria belonging to the **tuberculosis** complex (TC) or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitisation with antigens derived from mycobacteria belonging to the TC; or (c) an amino acid sequence having a sequence identity with (a) or the subsequence as in (b) of at least 70% and at the same time being immunologically equivalent to (I) with respect to the ability of evoking a protective immune response against infections with mycobacteria belonging to the TC or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitisation with antigens derived from mycobacteria belonging to the TC; provided that: (i) the polypeptide fragment is in pure form when consisting of the amino acid sequence 1-92 of Seq ID 2; or when consisting of the amino acid sequence 87-108 of Seq ID 4 fused to beta-galactosidase; (ii) the degree of sequence identity in (c) is at least 95% when the polypeptide comprises a homologue of a polypeptide which has the amino acid sequence of Seq ID 12 or a subsequence as in (b); and (iii) the polypeptide fragment contains a threonine residue corresponding to position 213 in Seq ID 42 when comprising an amino acid sequence of at least 6 amino acids in Seq ID 42. Also claimed are: (1) a fusion polypeptide fragment which comprises: (a) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from Mycobacterium **tuberculosis** (MT) protein **ESAT-6**, and a second amino acid sequence including at least one T-cell epitope derived from a MT protein different from **ESAT-6** and/or including a stretch of amino acids which protects the first amino acid sequence from in vivo degradation or post-translational processing; or (b) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the MT protein MPT59, and a second amino acid sequence including at least one T-cell epitope derived from a MT protein different from MPT59 and/or including a stretch of amino acids which protects the first amino acid sequence from in vivo degradation or post-translational processing; (2) a nucleic acid fragment in isolated form which: (a) comprises a nucleic acid sequence which encodes (I) or a polypeptide as in (1) or comprises a complementary nucleic acid sequence; (b) has a length of at least 10 nucleotides and hybridises readily under stringent hybridisation conditions with a nucleic acid fragment which has a nucleotide sequence (NS) selected from sequences 1, 3, 5, 7, 9, 11, 13, 15, 41, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 87, 89, 91, 93, 140, 142, 144, 146, 148, 150, and 152; or a complementary sequence, provided that when the nucleic acid fragment comprises a subsequence of Seq ID 41, then the nucleic acid fragment contains an A corresponding to position 781 in Seq ID 41, and when the nucleic acid fragment comprises a subsequence of a NS complementary to Seq ID 41, then the nucleic acid fragment comprises a T corresponding to position 781 in Seq ID 41; (3) a replicable expression vector which comprises a nucleic acid fragment as in (2); (4) a transformed cell harbouring at least one vector as in (3); (5) a monoclonal or polyclonal antibody which is specifically reactive with a (I) or a polypeptide as in (1).

USE The products can be used in the detection of and **prevention** of mycobacterial infections. In particular, the polypeptides and nucleic acids can be used for the diagnosis of or

vaccination against **tuberculosis** caused by MT, M africanum or M. bovis.
Dwg.0/6

L16 ANSWER 43 OF 47 WPIDS (C) 2003 THOMSON DERWENT
AN 1998-261042 [23] WPIDS
CR 1997-192903 [17]; 1998-251292 [22]; 1999-527409 [42]; 1999-601610 [51]; 2002-171134 [21]
DNN N1998-205794 DNC C1998-081032
TI Immunogenic Mycobacterium **tuberculosis** polypeptide(s) and DNA - used to develop products for the detection of M. **tuberculosis** infection and for diagnosis, **treatment** and **prevention** of **tuberculosis**.
DC B04 D16 S03
IN CAMPOS-NETO, A; DILLON, D C; HOUGHTON, R; LODES, M J; REED, S G; SKEIKY, Y A W; TWARDZIK, D R; VEDVICK, T S; SKEIKY, Y A
PA (CORI-N) CORIXA CORP
CYC 80
PI WO 9816646 A2 19980423 (199823)* EN 229p
RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU
ZA 9708969 A 19980624 (199831) 232p
AU 9748144 A 19980511 (199837)
NO 9901694 A 19990610 (199933)
EP 932681 A2 19990804 (199935) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
CZ 9901265 A3 19991117 (200002)
HU 9903475 A2 20000128 (200015)
CN 1241212 A 20000112 (200022)
BR 9712518 A 20001024 (200058)
MX 9903392 A1 19991201 (200110)
JP 2001501832 W 20010213 (200112) 245p
KR 2000049101 A 20000725 (200116)
US 6290969 B1 20010918 (200157)
ADT WO 9816646 A2 WO 1997-US18293 19971007; ZA 9708969 A ZA 1997-8969 19971007; AU 9748144 A AU 1997-48144 19971007; NO 9901694 A WO 1997-US18293 19971007, NO 1999-1694 19990409; EP 932681 A2 EP 1997-910873 19971007, WO 1997-US18293 19971007; CZ 9901265 A3 WO 1997-US18293 19971007, CZ 1999-1265 19971007; HU 9903475 A2 WO 1997-US18293 19971007, HU 1999-3475 19971007; CN 1241212 A CN 1997-180501 19971007; BR 9712518 A BR 1997-12518 19971007, WO 1997-US18293 19971007; MX 9903392 A1 MX 1999-3392 19990412; JP 2001501832 W WO 1997-US18293 19971007, JP 1998-518456 19971007; KR 2000049101 A WO 1997-US18293 19971007, KR 1999-703181 19990412; US 6290969 B1 CIP of US 1995-523436 19950901, CIP of US 1995-533634 19950922, CIP of US 1996-620874 19960322, CIP of US 1996-659683 19960605, CIP of US 1996-680574 19960712, CIP of US 1996-730510 19961011, US 1997-818112 19970313
FDT AU 9748144 A Based on WO 9816646; EP 932681 A2 Based on WO 9816646; CZ 9901265 A3 Based on WO 9816646; HU 9903475 A2 Based on WO 9816646; BR 9712518 A Based on WO 9816646; JP 2001501832 W Based on WO 9816646; KR 2000049101 A Based on WO 9816646
PRAI US 1997-818112 19970313; US 1996-730510 19961011; US 1995-523436 19950901; US 1995-533634 19950922; US 1996-620874 19960322; US 1996-659683 19960605; US 1996-680574 19960712
AB WO 9816646 A UPAB: 20020618
A polypeptide comprising an immunogenic portion of a soluble Mycobacterium **tuberculosis** (MT) antigen, or a variant of the antigen that differs only in conservative substitutions and/or modifications is new. The antigen has an N-terminal sequence selected from (I)-(XII), given below.

Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-Gln-Val-Val-Ala-Ala-Leu (I)

Ala-Val-Glu-Ser-Gly-Met-Leu-Ala-Leu-Gly-Thr-Pro-Ala-Pro-Ser (II)

Ala-Ala-Met-Lys-Pro-Arg-Thr-Gly-Asp-Gly-Pro-Leu-Glu-Ala-Ala-Lys-Glu-Gly-Arg (III)

Tyr-Tyr-Trp-Cys-Pro-Gly-Gln-Pro-Phe-Asp-Pro-Ala-Trp-Gly-Pro (IV)

Asp-Ile-Gly-Ser-Glu-Ser-Thr-Glu-Asp-Gln-Gln-Xaa-Ala-Val (V)

Ala-Glu-Glu-Ser-Ile-Ser-Thr-Xaa-Glu-Xaa-Ile-Val-Pro (VI)

Asp-Pro-Glu-Pro-Ala-Pro-Pro-Val-Pro-Thr-Thr-Ala-Ala-Ser-Pro-Pro-Ser (VII)

Ala-Pro-Lys-Thr-Tyr-Xaa-Glu-Glu-Leu-Lys-Gly-Thr-Asp-Thr-Gly (VIII)

Asp-Pro-Apa-Ser-Ala-Pro-Asp-Val-Pro-Thr-Ala-Ala-Gln-Leu-Thr-Ser-Leu-Leu-Asn-Ser-Leu-Ala-Asp-Pro-Asn-Val-Ser-Phe-Ala-Asn (IX)

Ala-Pro-Glu-Ser-Gly-Ala-Gly-Leu-Gly-Leu-Gly-Gly-Thr-Val-Gln-Ala-Gly (X)

Asp-Pro-Pro-Asp-Pro-His-Gln-Xaa-Asp-Met-Thr-Lys-Gly-Tyr-Tyr-Pro-Gly-Gly-Arg-Arg-Xaa-Phe (XI)

Xaa-Tyr-Ile-Ala-Tyr-Xaa-Thr-Thr-Ala Gly (XII)

Xaa = any amino acid.

Also claimed are: (1) a polypeptide as above that is encoded by one of seventy-five specified DNA sequences given in the specification; (2) a DNA molecule (I) as in (1); (3) an expression vector comprising (I); (4) a host cell transformed with an expression vector as in (3); (5) a fusion protein comprising: (a) two or more polypeptides described above; (b) one or more polypeptides as described above and **ESAT-6** (a previously identified antigen found in both M. bovis and M. **tuberculosis**; sequence given below) or the M. **tuberculosis** antigen 38 kD (374 amino acid sequence given in the specification):

Met Thr Glu Gln Gln Trp Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly Lys Gln Ser Leu Thr Lys Leu Ala Ala Trp Gly Gly Ser Gly Ser Glu Ala Tyr (**ESAT-6**); and

(6) a diagnostic kit comprising: (a) a polypeptide or fusion protein as described above, especially having an N-terminal sequence selected from: Xaa-Asp-Ser-Glu-Lys-Ser-Ala-Thr-Ile-Lys-Val-Thr-Asp-Ala-Ser; and Ala-Gly-Asp-Thr-Xaa-Ile-Tyr-Ile-Val-Gly-Asn-Leu-Thr-Ala-Asp; and (b) apparatus to contact the polypeptide or fusion protein with the dermal cells of a patient.

USE - The products can be used for the detection of MT infection for diagnosing **tuberculosis**. They can also be used to provide **vaccines** for **preventing** or **treating** **tuberculosis**.

Dwg.0/9

L16 ANSWER 44 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

AN 1998373720 EMBASE

TI Comparison of antigen-specific T-cell responses of **tuberculosis** patients using complex or single antigens of Mycobacterium **tuberculosis**.

AU Mustafa A.S.; Amoudy H.A.; Wiker H.G.; Abal A.T.; Ravn P.; Oftung F.; Andersen P.

CS A.S. Mustafa, Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait

SO Scandinavian Journal of Immunology, (1998) 48/5 (535-543).
Refs: 65
ISSN: 0300-9475 CODEN: SJIMAX

CY United Kingdom

DT Journal; Article

FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index

LA English

SL English

AB We have screened peripheral blood mononuclear cells (PBMC) from **tuberculosis** (TB) patients for proliferative reactivity and interferon-.gamma. (IFN-.gamma.) secretion against a panel of purified recombinant (r) and natural (n) culture filtrate (rESAT-6, nMPT59, nMPT64 and nMPB70) and somatic- derived (rGroES, rPstS, rGroEL and rDnaK) antigens of Mycobacterium **tuberculosis**. The responses of PBMC to these defined antigens were compared with the corresponding results obtained with complex antigens, such as whole- cell M. **tuberculosis**, M. **tuberculosis** culture filtrate (MT-CF) and cell wall antigens, as well as the **vaccine** strain, Mycobacterium bovis bacillus Calmette-Guerin (BCG). In addition, M. **tuberculosis** and MT-CF-induced T-cell lines were tested in the same assays against the panel of purified and complex antigens. The compiled data from PBMC and T-cell lines tested for antigen-induced proliferation and IFN-.gamma. secretion showed that the most frequently recognized antigen was **ESAT-6**, followed by MPT59, GroES, MPB70, MPT64, DnaK, GroEL and PstS. The frequency of **ESAT -6** responders, as measured both by proliferation (18/19) and secretion of IFN-.gamma. (16/19)was comparable to the results obtained with whole-cell M. **tuberculosis**, MT-CF and M. bovis BCG. We also observed that most of the high responders to complex antigens recognized all of the antigens tested (covariation), demonstrating that the repertoire of human T-cell specificities induced by natural infection is directed towards several unrelated culture filtrate as well as somatic-derived protein antigens. In conclusion, the results obtained suggest that the cellular immune response in humans is directed against several important target antigens of M. **tuberculosis** and that some antigens, such as **ESAT-6**, are recognized by a high number of individuals. Such antigens represent candidates to be used for development of specific diagnostic reagents or in subunit **vaccines**
.

L16 ANSWER 45 OF 47 MEDLINE
AN 1999069096 MEDLINE
DN 99069096 PubMed ID: 9791038
TI Cytotoxic T-lymphocytes against malaria and **tuberculosis**: from natural immunity to **vaccine** design.
AU Lalvani A; Hill A V
CS Nuffield Department of Clinical Medicine, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, U.K.
SO CLINICAL SCIENCE, (1998 Nov) 95 (5) 531-8.
Journal code: 7905731. ISSN: 0143-5221.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199901
ED Entered STN: 19990128
Last Updated on STN: 19990128
Entered Medline: 19990112
AB 1.Mycobacterium **tuberculosis** and the liver stage of Plasmodium falciparum are intracellular pathogens which are potentially susceptible to cytotoxic T-lymphocytes, a crucial component of the protective immune response to viral infections. Evidence from animal models points to a protective role for cytotoxic T-lymphocytes against M. **tuberculosis** and P. falciparum, but cytotoxic T-lymphocytes specific for these pathogens have been difficult to identify in man.2.Using a reverse immunogenetic approach, candidate epitopes from selected antigens of P. falciparum and M. **tuberculosis** were used to detect peptide-specific cytotoxic T-lymphocyte responses in individuals exposed to these pathogens. Cytotoxic T-lymphocyte activity was detected by the 51Cr release cytotoxicity assay and a sensitive ELISPOT assay for single-cell interferon-gamma release.3.In naturally exposed, partially

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18 8 DUP REM L17 (9 DUPLICATES REMOVED)

=> d bib ab 1-8

L18 ANSWER 1 OF 8 MEDLINE
AN 2003139705 MEDLINE
DN 22541529 PubMed ID: 12654816
TI Recognition of mycobacterial epitopes by T cells across mammalian species and use of a program that predicts human HLA-DR binding peptides to predict bovine epitopes.
AU Vordermeier Martin; Whelan Adam O; Hewinson R Glyn
CS TB Research Group, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom..
mvordermeier.vla@gtnet.gov.uk
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 1980-7.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB Bioinformatics tools have the potential to accelerate research into the design of vaccines and diagnostic tests by exploiting genome sequences. The aim of this study was to assess whether in silico analysis could be combined with in vitro screening methods to rapidly identify peptides that are immunogenic during Mycobacterium bovis infection of cattle. In the first instance the M. bovis-derived protein **ESAT-6** was used as a model antigen to describe peptides containing T-cell epitopes that were frequently recognized across mammalian species, including natural hosts for **tuberculosis** (humans and cattle) and small-animal models of **tuberculosis** (mice and guinea pigs). Having demonstrated that some peptides could be recognized by T cells from a number of M. bovis-infected hosts, we tested whether a virtual-matrix-based human prediction program (ProPred) could identify peptides that were recognized by T cells from M. bovis-infected cattle. In this study, 73% of the experimentally defined peptides from 10 M. bovis antigens that were recognized by bovine T cells contained motifs predicted by ProPred. Finally, in validating this observation, we showed that three of five peptides from the mycobacterial antigen Rv3019c that were predicted to contain HLA-DR-restricted epitopes were recognized by T cells from M. bovis-infected cattle. The results obtained in this study support the approach of using bioinformatics to increase the efficiency of epitope screening and selection.

L18 ANSWER 2 OF 8 MEDLINE
AN 2003084956 MEDLINE
DN 22477632 PubMed ID: 12588658
TI Human Th1 cell lines recognize the Mycobacterium **tuberculosis** **ESAT-6** antigen and its peptides in association with frequently expressed HLA class II molecules.
AU Mustafa A S; Shaban F A; Al-Attiyah R; Abal A T; El-Shamy A M; Andersen P; Oftung F
CS Department of Microbiology; Department of Medicine, Kuwait University, Safat; Chest Diseases Hospital, Kuwait.. abusalim@hs.kuniv.edu.kw
SO SCANDINAVIAN JOURNAL OF IMMUNOLOGY, (2003 Feb) 57 (2) 125-34.
Journal code: 0323767. ISSN: 0300-9475.
CY England: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200303
ED Entered STN: 20030225
Last Updated on STN: 20030314
Entered Medline: 20030313
AB We have used a synthetic-peptide approach to map epitope regions of the Mycobacterium **tuberculosis** **ESAT-6** antigen recognized by human T cells in relation to major histocompatibility complex (MHC) restriction. **ESAT-6**-specific CD4+ T-cell lines were established by stimulating peripheral blood mononuclear cells from 25 HLA-DR-typed **tuberculosis** patients with complete antigen in vitro. The established T-cell lines were then screened for proliferation and interferon-gamma (IFN-gamma) secretion in response to eight overlapping 20-mer peptides covering the **ESAT-6** sequence. The response of the T-cell lines to **ESAT-6** and peptides from a human leucocyte antigen (HLA)-heterogeneous group of donors suggested the presence of multiple epitopes and promiscuous recognition of the antigen. Analysis of antigen and peptide recognition in the presence of anti-HLA class I and class II antibodies suggested that the T-cell lines recognized **ESAT-6** in association with HLA-DR and -DQ molecules. Furthermore, testing of selected T-cell lines with **ESAT-6** and the peptides in the presence of autologous and allogeneic HLA-DR- and -DQ-typed antigen-presenting cells identified HLA-DR2, -DR52 and -DQ2 amongst the HLA molecules involved in the presentation of **ESAT-6** and its peptides to human Th1 cells. In addition, the T-cell lines were cytotoxic for monocytes and macrophages pulsed with **ESAT-6** and peptides. In conclusion, the recognition of **ESAT-6** by IFN-gamma-secreting and cytotoxic CD4+ T cells in association with frequently expressed HLA class II molecules supports the application of this antigen to either specific diagnosis or subunit vaccine design.

L18 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS
AN 2002:522161 CAPLUS
DN 137:88432
TI T cell level-based assay to determine efficacy of treatment for mycobacterial infection
IN Lalvani, Ajit
PA Isis Innovation Limited, UK
SO PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002054072	A2	20020711	WO 2002-GB55	20020108

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI GB 2001-432 A 20010108
US 2001-259868P P 20010108
AB The invention discloses a method for detg. the efficacy of treatment for mycobacterial infection in an individual, comprising detg. in samples from

EM 200301
ED Entered STN: 20021219
Last Updated on STN: 20030202
Entered Medline: 20030131
AB OBJECTIVES: An accurate test for Mycobacterium **tuberculosis** infection is urgently needed. The tuberculin skin test (TST) lacks sensitivity, particularly in HIV-infected individuals, and has poor specificity because of antigenic cross-reactivity with Bacillus Calmette-Guerin (BCG) vaccination. **ESAT-6** and CFP-10 are antigens expressed in Mycobacterium **tuberculosis**, but not in Mycobacterium bovis BCG and most environmental mycobacteria. We investigated whether T cells specific for these antigens could serve as accurate markers of M. **tuberculosis** infection in an area of high **tuberculosis** and HIV prevalence. METHODS: Using the rapid ex-vivo enzyme-linked immunospot (ELISPOT) assay for IFN-gamma, we enumerated T cells specific for **ESAT-6**, CFP-10 and purified protein derivative (PPD) in blood samples from 50 Zambian **tuberculosis** patients, 75 healthy Zambian adults, and 40 healthy UK residents. TSTs were performed in 49 healthy Zambian adults. RESULTS: All (100%; n = 11) and 90% (n = 39) of HIV-negative and HIV-positive **tuberculosis** patients, respectively, had detectable **ESAT-6**- or CFP-10-specific T cells. The **ESAT-6**/CFP-10-based ELISPOT assay was positive in 37 out of 54 HIV-negative healthy Zambians, suggesting a 69% prevalence of latent M. **tuberculosis** infection. Fewer HIV-positive Zambians possessed **ESAT-6** /CFP-10-specific T cells, but the impact of HIV infection was less on this assay than on the PPD-based ELISPOT or TST. CONCLUSION: The **ESAT -6**/CFP-10-based ELISPOT assay detects active **tuberculosis** in HIV-positive individuals with high sensitivity. It is more specific, and possibly more sensitive, than PPD-based methods of detecting latent M. **tuberculosis** infection, and may potentially improve the targeting of isoniazid preventative therapy to HIV-positive individuals with latent **tuberculosis** infection.

L18 ANSWER 6 OF 8 MEDLINE
AN 2001125769 MEDLINE
DN 21064969 PubMed ID: 11133379
TI Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium **tuberculosis** infection in healthy urban Indians.
CM Comment in: J Infect Dis. 2001 Dec 1;184(11):1497-8
AU Lalvani A; Nagvenkar P; Udwadia Z; Pathan A A; Wilkinson K A; Shastri J S; Ewer K; Hill A V; Mehta A; Rodrigues C
CS Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.. ajit.lalvani@ndm.ox.ac.uk
SO JOURNAL OF INFECTIOUS DISEASES, (2001 Feb 1) 183 (3) 469-77.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200102
ED Entered STN: 20010322
Last Updated on STN: 20030105
Entered Medline: 20010222
AB Knowledge of the prevalence of latent Mycobacterium **tuberculosis** infection is crucial for effective **tuberculosis** control, but tuberculin skin test surveys have major limitations, including poor specificity because of the broad antigenic cross-reactivity of tuberculin. The M. **tuberculosis** RD1 genomic segment encodes proteins, such as early secretory antigenic target (**ESAT**)-**6**, that are absent from M. bovis bacille Calmette-Guerin (BCG) and most environmental

expressed a fusion protein, while rBCG-2, with a secretory sequence, could secret ESAT-6 into the culture medium. There was no evidence for increased virulence of the two rBCG strains when we made a comparison between them and BCG with regard to organ bacterial loads, lung histology, and survival time. rBCG-1 induced significantly higher specific antibody titers and stronger cellular immune response than BCG, whereas rBCG-2 had immunogenicity similar to that of the parental BCG strain. Both rBCG-1 and rBCG-2 conferred marked protection against M. **tuberculosis** infection, yet in terms of protective efficacy, they showed no significant improvements upon conventional BCG vaccine.

L22 ANSWER 4 OF 28 MEDLINE
AN 2003195783 MEDLINE
DN 22554816 PubMed ID: 12667217
TI A DNA prime-live vaccine boost strategy in mice can augment IFN-gamma responses to mycobacterial antigens but does not increase the protective efficacy of two attenuated strains of Mycobacterium bovis against bovine **tuberculosis**.
AU Skinner M A; Ramsay A J; Buchan G S; Keen D L; Ranasinghe C; Slobbe L; Collins D M; de Lisle G W; Buddle B M
CS AgResearch Ltd, Wallaceville Animal Research Centre, Upper Hutt, New Zealand.. margot.skinner@agresearch.co.nz
SO IMMUNOLOGY, (2003 Apr) 108 (4) 548-55.
Journal code: 0374672. ISSN: 0019-2805.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200306
ED Entered STN: 20030429
Last Updated on STN: 20030608
Entered Medline: 20030606
AB The Mycobacterium bovis bacille Calmette-Guerin (BCG) vaccine has variable efficacy for both human and bovine **tuberculosis**. There is a need for improved vaccines or vaccine strategies for control of these diseases. A recently developed prime-boost strategy was investigated for vaccination against M. bovis infection in mice. BALB/c and C57BL/6 mice were primed with a DNA vaccine, expressing two mycobacterial antigens, ESAT-6 and antigen 85 A and boosted with attenuated M. bovis strains, BCG or WAg520, a newly attenuated strain, prior to aerosol challenge. Before challenge, the antigen-specific production of interferon-gamma (IFN-gamma) was evaluated by ELISPOT and antibody responses were measured. The prime-boost stimulated an increase in the numbers of IFN-gamma producing cells compared with DNA or live vaccination alone, but this varied according to the attenuated vaccine strain, time of challenge and the strain of mouse used. Animals vaccinated with DNA alone generated the strongest antibody response to mycobacterial antigens, which was predominantly IgG1. BCG and WAg520 alone generally gave a 1-2 log10 reduction in bacterial load in lungs or spleen, compared to non-vaccinated or plasmid DNA only control groups. The prime-boost regimen was not more effective than BCG or WAg520 alone. These observations demonstrate the comparable efficacy of BCG and WAg520 in a mouse model of bovine **tuberculosis**. However, priming with the DNA vaccine and boosting with an attenuated M. bovis vaccine enhanced IFN-gamma immune responses compared to vaccinating with an attenuated M. bovis vaccine alone, but did not increase protection against a virulent M. bovis infection.

L22 ANSWER 5 OF 28 MEDLINE DUPLICATE 1
AN 2003204563 MEDLINE
DN 22610413 PubMed ID: 12692540
TI Recombinant BCG exporting ESAT-6 confers enhanced protection against **tuberculosis**.
CM Comment in: Nat Med. 2003 May;9(5):503-4

AU Pym Alexander S; Brodin Priscille; Majlessi Laleh; Brosch Roland; Demangel Caroline; Williams Ann; Griffiths Karen E; Marchal Gilles; Leclerc Claude; Cole Stewart T
CS Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France.
SO NATURE MEDICINE, (2003 May) 9 (5) 533-9.
Journal code: 9502015. ISSN: 1078-8956.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200306
ED Entered STN: 20030502
Last Updated on STN: 20030627
Entered Medline: 20030626
AB The live **tuberculosis** vaccines Mycobacterium bovis BCG (bacille Calmette-Guerin) and Mycobacterium microti both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa **early secretory antigenic target**) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the Mycobacterium **tuberculosis** complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the esxA and esxB genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with M. **tuberculosis**, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.

L22 ANSWER 6 OF 28 MEDLINE
AN 2003012064 MEDLINE
DN 22406403 PubMed ID: 12518231
TI Combined recombinant DNA vaccine results in significant protection against Mycobacterium **tuberculosis**.
AU Pan Yi; Cai Hong; Li Shu-Xia; Tian Xia; Li Tang; Zhu Yu-Xian
CS College of Life Sciences, Peking University, Beijing 100871, China.
SO Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai), (2003 Jan) 35 (1) 71-6.
Journal code: 20730160R. ISSN: 0582-9879.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200305
ED Entered STN: 20030109
Last Updated on STN: 20030522
Entered Medline: 20030521
AB Three proteins secreted from Mycobacterium **tuberculosis**, Ag85B, ESAT-6 and MPT63 were selected as antigens for making combined DNA vaccine by immunizing mice. The immune response induced by the vaccine and its protective efficacy were studied. It was demonstrated that when mice were immunized with the combined DNA vaccine, the titer of antibody for Ag85B in serum increased to more than 10(5), but the titers of ESAT-6 and MPT63 specific antibodies were undetectable. After the final immunization, the level of gamma specific for Ag85B, ESAT-6 and MPT63 reached (17.0+/-7.0) u/ml, (6.0+/-0.8) u/ml and (11.9+/-8.0) u/ml, respectively. Mice, that were inoculated with the empty eukaryotic expression vector pJW4303 DNA, produced negligible amounts of antigen-specific INF-gamma. The combined

DNA vaccine resulted also in significantly reduced amount of bacteria in the lungs of experimental mice. Microphotographs showed clearly that these lungs were better protected against Mycobacterium **tuberculosis** challenge than control mice. The combined DNA vaccine reported in this work shed new lights on the prophylactic protection against **tuberculosis**.

L22 ANSWER 7 OF 28 MEDLINE
AN 2002271828 MEDLINE
DN 22006918 PubMed ID: 12011005
TI Oral vaccination with subunit vaccines protects animals against aerosol infection with Mycobacterium **tuberculosis**.
AU Doherty T Mark; Olsen Anja Weinrich; van Pinxteren Laurens; Andersen Peter
CS Department of Tuberculosis Immunology, Statens Serum Institute, Copenhagen, Denmark.. markdoc@hotmail.com
SO INFECTION AND IMMUNITY, (2002 Jun) 70 (6) 3111-21.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200206
ED Entered STN: 20020516
Last Updated on STN: 20030111
Entered Medline: 20020626
AB Immunity against Mycobacterium **tuberculosis** depends largely on activation of cell-mediated responses, and gamma interferon has been shown to play a crucial role in this process in both humans and animal models. Since the lung is normally the organ in which infection is initiated and is the major site of pathology, immune responses in the lung play a significant role in restricting initial infection with M. **tuberculosis**. The aim of the present study was to stimulate efficient immunity in the lung by targeting the gut mucosa. Detoxified monophosphoryl lipid A (MPL) has been shown to be a relatively nontoxic adjuvant which efficiently promotes the induction of type 1 responses when it is given by the traditional subcutaneous route. We have therefore compared subcutaneous immunization of mice to oral immunization by using a model subunit vaccine carrying two immunodominant proteins from M. **tuberculosis**, in combination with MPL-based adjuvants. While less effective when used to prime a response, a heterologous priming and boosting vaccination strategy employing oral boosting induced significant systemic type 1 responses which equaled and surpassed those attained by subcutaneous immunization protocols. Moreover, the increased immune responses observed correlated with the induction of substantial protection against subsequent aerosol infection with virulent M. **tuberculosis** at levels comparable to, or better than, those obtained by multiple subcutaneous vaccinations. These results demonstrate that booster vaccinations via mucosal surfaces, by combining efficient subunit vaccines with the potent adjuvant MPL, may be an effective method of addressing some of the shortcomings of current vaccination strategies.

L22 ANSWER 8 OF 28 MEDLINE
AN 2002271820 MEDLINE
DN 22006907 PubMed ID: 12010994
TI Correlation of ESAT-6-specific gamma interferon production with pathology in cattle following Mycobacterium bovis BCG vaccination against experimental bovine **tuberculosis**.
AU Vordermeier H Martin; Chambers Mark A; Cockle Paul J; Whelan Adam O; Simmons Jennifer; Hewinson R Glyn
CS Veterinary Laboratories Agency Weybridge, TB Research Group, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.. mvordermeier.vla@gtnet.gov.uk
SO INFECTION AND IMMUNITY, (2002 Jun) 70 (6) 3026-32.

Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200206
ED Entered STN: 20020516
Last Updated on STN: 20020627
Entered Medline: 20020626
AB Vaccine development and the understanding of the pathology of bovine **tuberculosis** in cattle would be greatly facilitated by the definition of immunological correlates of protection and/or pathology. To address these questions, cattle were vaccinated with Mycobacterium bovis bacillus Calmette-Guerin (BCG) and were then challenged with virulent M. bovis. Applying a semiquantitative pathology-scoring system, we were able to demonstrate that BCG vaccination imparted significant protection by reducing the disease severity on average by 75%. Analysis of cellular immune responses following M. bovis challenge demonstrated that proliferative T-cell and gamma interferon (IFN-gamma) responses towards the M. bovis-specific antigen ESAT-6, whose gene is absent from BCG, were generally low in vaccinated animals but were high in all nonvaccinated calves. Importantly, the amount of ESAT-6-specific IFN-gamma measured by enzyme-linked immunosorbent assay after M. bovis challenge, but not the frequency of responding cells, correlated positively with the degree of pathology found 18 weeks after infection. Diagnostic reagents based on antigens not present in BCG, like ESAT-6 and CFP-10, were still able to distinguish BCG-vaccinated, diseased animals from BCG-vaccinated animals without signs of disease. In summary, our results suggest that the determination of ESAT-6-specific IFN-gamma, while not a direct correlate of protection, constitutes nevertheless a useful prognostic immunological marker predicting both vaccine efficacy and disease severity.

L22 ANSWER 9 OF 28 MEDLINE
AN 2002084248 MEDLINE
DN 21655197 PubMed ID: 11796642
TI Antigenic specificity of the Mycobacterium leprae homologue of ESAT-6.
AU Spencer John S; Marques Maria Angela M; Lima Monica C B S; Junqueira-Kipnis Ana Paula; Gregory Bruce C; Truman Richard W; Brennan Patrick J
CS Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523-1677, USA.. john.spencer@colostate.edu
NC NO1 AI-55262 (NIAID)
NO1 AI-75320 (NIAID)
SO INFECTION AND IMMUNITY, (2002 Feb) 70 (2) 1010-3.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200202
ED Entered STN: 20020129
Last Updated on STN: 20020222
Entered Medline: 20020221
AB The sequence of the Mycobacterium leprae homologue of ESAT-6 shows only 36% amino acid correspondence to that from Mycobacterium **tuberculosis**. Anti-M. leprae ESAT-6 polyclonal and monoclonal antibodies and T-cell hybridomas reacted only with the homologous protein and allowed identification of the B- and T-cell epitopes. The protein is expressed in M. leprae and appears in the cell wall fraction. Thus, M. leprae ESAT-6 shows promise as a specific diagnostic agent for leprosy.

L22 ANSWER 10 OF 28 MEDLINE
AN 2002082254 MEDLINE

bacillus. To quantitate M. **tuberculosis**-specific T cells directly ex vivo, we enumerated IFN-gamma-secreting CD4 T cells specific for ESAT-6, a secreted Ag that is highly specific for M. **tuberculosis**, and a target of protective immune responses in animal models. We found that frequencies of circulating ESAT-6 peptide-specific IFN-gamma-secreting CD4 T cells were higher in latently infected healthy contacts and subjects with minimal disease and low bacterial burdens than in patients with culture-positive active pulmonary **tuberculosis** ($p = 0.009$ and $p = 0.002$, respectively). Importantly, the frequency of these Ag-specific CD4 T cells fell progressively in all groups with **treatment** ($p = 0.005$), suggesting that the lower responses in patients with more extensive disease were not due to **tuberculosis**-induced immune suppression. This population of M. **tuberculosis** Ag-specific Th1-type CD4 T cells appears to correlate with clinical phenotype and declines during successful therapy; these features are consistent with a role for these T cells in the containment of M. **tuberculosis** in vivo. Such findings may assist in the design and evaluation of novel **tuberculosis** vaccine candidates.

L22 ANSWER 14 OF 28 MEDLINE
AN 2001366967 MEDLINE
DN 21321119 PubMed ID: 11427279
TI Protective efficacy against **tuberculosis** of ESAT-6 secreted by a live Salmonella typhimurium vaccine carrier strain and expressed by naked DNA.
AU Mollenkopf H J; Groine-Triebkorn D; Andersen P; Hess J; Kaufmann S H
CS Max-Planck-Institute for Infection Biology, Department of Immunology, Schumannstr. 21/22, 10117 Berlin, Germany.. mollenkopf@mpiib-berlin.mpg.de
SO VACCINE, (2001 Jul 16) 19 (28-29) 4028-35.
Journal code: 8406899. ISSN: 0264-410X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20011015
Last Updated on STN: 20011015
Entered Medline: 20011011
AB We have constructed a recombinant (r) attenuated Salmonella typhimurium strain which secretes ESAT-6 of Mycobacterium **tuberculosis** via the hemolysin secretion system of E. coli. Additionally, we have ligated ESAT-6 to different commercially available mammalian expression systems for use as naked DNA vaccines. We studied protection against M. **tuberculosis** induced by vaccination with each of these constructs alone or in combination in mice. Vaccination with a single dose of r S. typhimurium secreting ESAT-6 reduced numbers of tubercle bacilli in the lungs throughout the course of infection. The combined prime-boost vaccination did not considerably enhance protection.

L22 ANSWER 15 OF 28 MEDLINE
AN 2001248071 MEDLINE
DN 21189184 PubMed ID: 11292688
TI Protection of mice with a **tuberculosis** subunit vaccine based on a fusion protein of antigen 85b and esat-6.
AU Weinrich Olsen A; van Pinxteren L A; Meng Okkels L; Birk Rasmussen P; Andersen P
CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (2001 May) 69 (5) 2773-8.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals
EM 200105
ED Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510
AB In this study, we investigated the potential of a **tuberculosis** subunit vaccine based on fusion proteins of the immunodominant antigens ESAT-6 and antigen 85B. When the fusion proteins were administered to mice in the adjuvant combination dimethyl dioctadecylammonium bromide-monophosphoryl lipid A, a strong dose-dependent immune response was induced to both single components as well as to the fusion proteins. The immune response induced was accompanied by high levels of protective immunity and reached the level of Mycobacterium bovis BCG-induced protection over a broad dose range. The vaccine induced efficient immunological memory, which remained stable 30 weeks postvaccination.

L22 ANSWER 16 OF 28 MEDLINE
AN 2002024114 MEDLINE
DN 21360002 PubMed ID: 11467375
TI Uncommon presentations of **tuberculosis**: the potential value of a novel diagnostic assay based on the Mycobacterium **tuberculosis** -specific antigens ESAT-6 and CFP-10.
AU Arend S M; Ottenhoff T H; Andersen P; van Dissel J T
CS Department of Infectious Diseases, Leiden University Medical Center, The Netherlands.. s.m.arend@lumc.nl
SO INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE, (2001 Jul) 5 (7) 680-6.
Journal code: 9706389. ISSN: 1027-3719.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011205
AB SETTING: Leiden University Medical Center, Leiden, the Netherlands. OBJECTIVE: To illustrate the potential value of a recently developed diagnostic assay for detection of **tuberculosis** (TB), based on T cell responses to the early secreted antigenic target 6 kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are Mycobacterium **tuberculosis** specific because they are expressed by M. **tuberculosis** but absent from M. bovis bacille Calmette-Guerin (BCG) and most environmental mycobacteria. In recent studies, the assay had a high sensitivity and specificity for detection of active TB. DESIGN: We describe five patients with uncommon presentations of **tuberculosis**, in whom the diagnosis was delayed by negative or conflicting results of diagnostic procedures aimed at detection of M. **tuberculosis** and an uninformative tuberculin skin test. IFN-gamma production in response to ESAT-6 and CFP-10 by peripheral blood mononuclear cells from these patients was evaluated before and during anti-**tuberculosis treatment**. RESULTS: In all five patients, IFN-gamma responses to ESAT-6 and/or CFP-10 were above the cut-off level defined in a previous study. During **treatment**, IFN-gamma responses generally increased. CONCLUSION: These results indicate that T cell responses to M. **tuberculosis**-specific antigens have potential diagnostic value when TB is suspected and the results of other diagnostic tests are inconclusive, especially in BCG-vaccinated individuals.

L22 ANSWER 17 OF 28 MEDLINE
AN 2001566195 MEDLINE
DN 21525390 PubMed ID: 11669220

TI Antigen discovery and **tuberculosis** vaccine development in the post-genomic era.
AU Louise R; Skjot V; Agger E M; Andersen P
CS Department of TB Immunology, Statens Serum Institut, Copenhagen, Denmark.
SO SCANDINAVIAN JOURNAL OF INFECTIOUS DISEASES, (2001) 33 (9) 643-7. Ref: 43
Journal code: 0215333. ISSN: 0036-5548.
CY Sweden
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20011024
Last Updated on STN: 20020302
Entered Medline: 20020301
AB For a number of years, a major effort has been put into the identification of candidate molecules for inclusion in a novel vaccine against **tuberculosis**. Various techniques have been exploited and have resulted in the identification of immunologically important antigens such as the immunodominant antigens ESAT-6 and antigen 85A/B. Today, the availability of the total nucleotide sequence of the Mycobacterium **tuberculosis** genome enables a post-genomic antigen discovery approach based on denotation and screening of complete protein families containing immunodominant molecules. One group of genes sharing properties with ESAT-6 constitute what has been called the esat-6 gene family. The genes have 10-35% homology to esat-6, are approximately the same size and share genomic organization. The data accumulated so far demonstrate that these molecules are immunodominant antigens strongly recognized in human TB patients and with the potential for a novel TB vaccine.

L22 ANSWER 18 OF 28 MEDLINE
AN 2001410275 MEDLINE
DN 21229296 PubMed ID: 11329460
TI Use of synthetic peptides derived from the antigens ESAT-6 and CFP-10 for differential diagnosis of bovine **tuberculosis** in cattle.
AU Vordermeier H M; Whelan A; Cockle P J; Farrant L; Palmer N; Hewinson R G
CS TB Research Group, Department of Bacterial Diseases, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone KT15 3NB, United Kingdom.. mvordermeier.vla@gtnet.gov.uk
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2001 May) 8 (3) 571-8.
Journal code: 9421292. ISSN: 1071-412X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200107
ED Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719
AB In Great Britain an independent scientific review for the government has concluded that the development of a cattle vaccine against Mycobacterium bovis infection holds the best long-term prospect for **tuberculosis** control in British herds. A precondition for vaccination is the development of a complementary diagnostic test to differentiate between vaccinated animals and those infected with M. bovis so that testing and slaughter-based control strategies can continue alongside vaccination. To date bacillus Calmette-Guerin (BCG), an attenuated strain of M. bovis, is the only available vaccine for the **prevention** of **tuberculosis**. However, tests based on tuberculin purified protein derivative cannot distinguish between M. bovis infection and BCG vaccination. Therefore, specific antigens expressed by M. bovis but

EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001222
AB SETTING: Strains of the Mycobacterium **tuberculosis** complex are being rationally attenuated in order to develop better **tuberculosis** vaccines than BCG, and it would be helpful if new vaccines lacked an immunogenic protein which could be used as a skin test reagent for determining infection status. OBJECTIVE: To delete the esat6 gene from a virulent Mycobacterium bovis strain and determine (i) whether this mutant sensitizes guinea pigs to a skin test based on ESAT6 and (ii) what effect this has on the virulence of M. bovis. DESIGN: An homologous recombination technique was used to produce an esat6 knockout mutant of a virulent strain of M. bovis. Guinea pigs were inoculated with either the mutant or parent strain and their reactivity in intradermal skin tests was determined to bovine purified protein derivative (PPD) and recombinant ESAT6 protein. RESULTS: Production of an esat6 knockout strain was demonstrated by Southern blot hybridization and the polymerase chain reaction. Guinea pigs inoculated with either the esat6 knockout strain or its virulent parent had positive skin test reactions to PPD but only animal inoculated with the parent strain had positive skin test reactions to ESAT6. Gross pathology, histopathology and mycobacterial culture of tissues indicated that the knockout strain was less virulent than its parent. CONCLUSION: If an effective live **tuberculosis** vaccine can be produced by inactivation of virulence genes in M. bovis, then prior or subsequent knockout of the esat6 gene could contribute to the loss of virulence and enable the development of a test to distinguish between vaccinated and infected animals.

L22 ANSWER 26 OF 28 MEDLINE
AN 1999386877 MEDLINE
DN 99386877 PubMed ID: 10456931
TI Immunogenicity of DNA vaccines expressing **tuberculosis** proteins fused to tissue plasminogen activator signal sequences.
AU Li Z; Howard A; Kelley C; Delogu G; Collins F; Morris S
CS Laboratory of Mycobacteria, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892, USA.
SO INFECTION AND IMMUNITY, (1999 Sep) 67 (9) 4780-6.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
ED Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991005
AB Novel **tuberculosis** DNA vaccines encoding native ESAT-6, MPT-64, KatG, or HBHA mycobacterial proteins or the same proteins fused to tissue plasminogen activator (TPA) signal sequences were evaluated for their capacity to elicit humoral, cell-mediated, and protective immune responses in vaccinated mice. While all eight plasmids induced specific humoral responses, the constructs expressing the TPA fusions generally evoked higher antibody responses in vaccinated hosts. Although most of the DNA vaccines tested induced a substantial gamma interferon response in the spleen, the antigen-specific lung responses were 2- to 10-fold lower than the splenic responses at the time of challenge. DNA vaccines encoding the ESAT-6, MPT-64, and KatG antigens fused to TPA signal sequences evoked significant protective responses in mice aerogenically challenged with low doses of Mycobacterium **tuberculosis** Erdman 17 to 21 days after the final immunization. However, the protective response induced by live Mycobacterium bovis BCG vaccine was greater than the response induced by

any of the DNA vaccines tested. These results suggest that the **tuberculosis** DNA vaccines were able to elicit substantial immune responses in suitably vaccinated mice, but further refinements to the constructs or the use of alternative immunization strategies will be needed to improve the efficacy of these vaccine candidates.

L22 ANSWER 27 OF 28 MEDLINE
AN 1999184991 MEDLINE
DN 99184991 PubMed ID: 10085007
TI Differential protective efficacy of DNA vaccines expressing secreted proteins of Mycobacterium **tuberculosis**.
AU Kamath A T; Feng C G; Macdonald M; Briscoe H; Britton W J
CS Centenary Institute of Cancer Medicine and Cell Biology, Newtown, New South Wales 2042, Australia.
SO INFECTION AND IMMUNITY, (1999 Apr) 67 (4) 1702-7.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990511
Last Updated on STN: 20030111
Entered Medline: 19990426
AB The development of more-effective antituberculosis vaccines would assist in the control of the global problem of infection with Mycobacterium **tuberculosis**. One recently devised vaccination strategy is immunization with DNA plasmids encoding individual microbial genes. Using the genes for the M. **tuberculosis** secreted proteins MPT64 (23 kDa), Ag85B (30 kDa), and ESAT-6 (6 kDa) as candidate antigens, DNA vaccines were prepared and tested for immunogenicity and protective efficacy in a murine model of aerosolized **tuberculosis** (TB). Intramuscular immunization with DNA-64 or DNA-85B resulted in the activation of CD4(+) T cells, which produce gamma interferon (IFN-gamma), and high titers of specific immunoglobulin G antibodies. Further, DNA-64 induced major histocompatibility complex class I-restricted CD8(+) cytotoxic T cells. The addition of a eukaryotic leader sequence to mpt64 did not significantly increase the T-cell or antibody response. Each of the three DNA vectors stimulated a significant reduction in the level of M. **tuberculosis** infection in the lungs of mice challenged 4 weeks after immunization, but not to the levels resulting after immunization with Mycobacterium bovis BCG. The vaccines showed a consistent hierarchy of protection, with the most effective being Ag85B, followed by ESAT-6 and then MPT64. Coimmunization with the three vectors resulted in a greater degree of protection than that induced by any single vector. This protective efficacy was associated with the emergence of IFN-gamma-secreting T cells earlier than in infected animals immunized with a control vector. The efficacy of these DNA vaccines suggests that multisubunit vaccination may contribute to future vaccine strategies against TB.

L22 ANSWER 28 OF 28 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 1998373720 EMBASE
TI Comparison of antigen-specific T-cell responses of **tuberculosis** patients using complex or single antigens of Mycobacterium **tuberculosis**.
AU Mustafa A.S.; Amoudy H.A.; Wiker H.G.; Abal A.T.; Ravn P.; Oftung F.; Andersen P.
CS A.S. Mustafa, Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait
SO Scandinavian Journal of Immunology, (1998) 48/5 (535-543).
Refs: 65
ISSN: 0300-9475 CODEN: SJIMAX

CY United Kingdom
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB We have screened peripheral blood mononuclear cells (PBMC) from **tuberculosis** (TB) patients for proliferative reactivity and interferon-.gamma. (IFN-.gamma.) secretion against a panel of purified recombinant (r) and natural (n) culture filtrate (rESAT-6, nMPT59, nMPT64 and nMPB70) and somatic- derived (rGroES, rPstS, rGroEL and rDnaK) antigens of Mycobacterium **tuberculosis**. The responses of PBMC to these defined antigens were compared with the corresponding results obtained with complex antigens, such as whole- cell M. **tuberculosis**, M. **tuberculosis** culture filtrate (MT-CF) and cell wall antigens, as well as the vaccine strain, Mycobacterium bovis bacillus Calmette-Guerin (BCG). In addition, M. **tuberculosis** and MT-CF-induced T-cell lines were tested in the same assays against the panel of purified and complex antigens. The compiled data from PBMC and T-cell lines tested for antigen-induced proliferation and IFN-.gamma. secretion showed that the most frequently recognized antigen was ESAT-6, followed by MPT59, GroES, MPB70, MPT64, DnaK, GroEL and PstS. The frequency of ESAT-6 responders, as measured both by proliferation (18/19) and secretion of IFN-.gamma. (16/19)was comparable to the results obtained with whole-cell M. **tuberculosis**, MT-CF and M. bovis BCG. We also observed that most of the high responders to complex antigens recognized all of the antigens tested (covariation), demonstrating that the repertoire of human T-cell specificities induced by natural infection is directed towards several unrelated culture filtrate as well as somatic-derived protein antigens. In conclusion, the results obtained suggest that the cellular immune response in humans is directed against several important target antigens of M. **tuberculosis** and that some antigens, such as ESAT-6, are recognized by a high number of individuals. Such antigens represent candidates to be used for development of specific diagnostic reagents or in subunit vaccines.

=> d his

(FILE 'HOME' ENTERED AT 12:28:34 ON 17 JUL 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 12:28:50 ON 17 JUL 2003

```
                E LALVANI AJIT/AU
L1          152 S E1-E3
                E PATHAN ANSAR/AU
L2           33 S E1-E5
L3          169 S L1-L2
L4           38 S L3 AND ESAT-6
L5            0 S L4 AND (ES1 OR ES2 OR ES3)
L6           12 DUP REM L4 (26 DUPLICATES REMOVED)
L7          583 S ESAT-6
L8          564 S L7 AND TUBERCULOSIS
L9           11 S L8 AND T CELL RECOGNI?
L10           3 DUP REM L9 (8 DUPLICATES REMOVED)
L11          35 S L8 AND T CELL (5A) RECOGNI?
L12          20 S L11 AND (DIAGNOSIS OR DIAGNOSTIC OR ASSAY OR DETECT?)
L13           7 DUP REM L12 (13 DUPLICATES REMOVED)
L14         382 S L8 AND VACCIN?
L15          69 S L14 AND (TREATING OR TREATMENT OR PREVENTING OR PREVENTION)
L16          47 DUP REM L15 (22 DUPLICATES REMOVED)
L17          17 S L8 AND EPITOP? (5A) MAPPING
```

L18 8 DUP REM L17 (9 DUPLICATES REMOVED)
L19 123 S EARLY SECRETORY ANTIGENIC TARGET?
L20 120 S L19 AND TUBERCULOSIS
L21 30 S L20 AND (TREATING OR TREATMENT OR PREVENTING OR PREVENTION)
L22 28 DUP REM L21 (2 DUPLICATES REMOVED)

=> s l20 and t cell (5a) recogni?
L23 6 L20 AND T CELL (5A) RECOGNI?

=> dup rem l23
PROCESSING COMPLETED FOR L23
L24 5 DUP REM L23 (1 DUPLICATE REMOVED)

=> d bib ab 1-5

L24 ANSWER 1 OF 5 MEDLINE
AN 2003139705 MEDLINE
DN 22541529 PubMed ID: 12654816
TI Recognition of mycobacterial epitopes by T cells across mammalian species and use of a program that predicts human HLA-DR binding peptides to predict bovine epitopes.
AU Vordermeier Martin; Whelan Adam O; Hewinson R Glyn
CS TB Research Group, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom..
mvordermeier.vla@gtnet.gov.uk
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 1980-7.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB Bioinformatics tools have the potential to accelerate research into the design of vaccines and diagnostic tests by exploiting genome sequences. The aim of this study was to assess whether in silico analysis could be combined with in vitro screening methods to rapidly identify peptides that are immunogenic during Mycobacterium bovis infection of cattle. In the first instance the M. bovis-derived protein ESAT-6 was used as a model antigen to describe peptides containing **T-cell** epitopes that were frequently **recognized** across mammalian species, including natural hosts for **tuberculosis** (humans and cattle) and small-animal models of **tuberculosis** (mice and guinea pigs). Having demonstrated that some peptides could be recognized by T cells from a number of M. bovis-infected hosts, we tested whether a virtual-matrix-based human prediction program (ProPred) could identify peptides that were recognized by T cells from M. bovis-infected cattle. In this study, 73% of the experimentally defined peptides from 10 M. bovis antigens that were recognized by bovine T cells contained motifs predicted by ProPred. Finally, in validating this observation, we showed that three of five peptides from the mycobacterial antigen Rv3019c that were predicted to contain HLA-DR-restricted epitopes were recognized by T cells from M. bovis-infected cattle. The results obtained in this study support the approach of using bioinformatics to increase the efficiency of epitope screening and selection.

L24 ANSWER 2 OF 5 MEDLINE
AN 2003084956 MEDLINE
DN 22477632 PubMed ID: 12588658
TI Human Th1 cell lines recognize the Mycobacterium **tuberculosis**

ESAT-6 antigen and its peptides in association with frequently expressed HLA class II molecules.

AU Mustafa A S; Shaban F A; Al-Attiyah R; Abal A T; El-Shamy A M; Andersen P; Oftung F

CS Department of Microbiology; Department of Medicine, Kuwait University, Safat; Chest Diseases Hospital, Kuwait.. abusalim@hs.kuniv.edu.kw

SO SCANDINAVIAN JOURNAL OF IMMUNOLOGY, (2003 Feb) 57 (2) 125-34. Journal code: 0323767. ISSN: 0300-9475.

CY England: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200303

ED Entered STN: 20030225
Last Updated on STN: 20030314
Entered Medline: 20030313

AB We have used a synthetic-peptide approach to map epitope regions of the Mycobacterium **tuberculosis** ESAT-6 antigen recognized by human T cells in relation to major histocompatibility complex (MHC) restriction. ESAT-6-specific CD4+ T-cell lines were established by stimulating peripheral blood mononuclear cells from 25 HLA-DR-typed **tuberculosis** patients with complete antigen in vitro. The established T-cell lines were then screened for proliferation and interferon-gamma (IFN-gamma) secretion in response to eight overlapping 20-mer peptides covering the ESAT-6 sequence. The response of the T-cell lines to ESAT-6 and peptides from a human leucocyte antigen (HLA)-heterogeneous group of donors suggested the presence of multiple epitopes and promiscuous recognition of the antigen. Analysis of antigen and peptide recognition in the presence of anti-HLA class I and class II antibodies suggested that the **T-cell** lines **recognized** ESAT-6 in association with HLA-DR and -DQ molecules. Furthermore, testing of selected T-cell lines with ESAT-6 and the peptides in the presence of autologous and allogeneic HLA-DR- and -DQ-typed antigen-presenting cells identified HLA-DR2, -DR52 and -DQ2 amongst the HLA molecules involved in the presentation of ESAT-6 and its peptides to human Th1 cells. In addition, the T-cell lines were cytotoxic for monocytes and macrophages pulsed with ESAT-6 and peptides. In conclusion, the recognition of ESAT-6 by IFN-gamma-secreting and cytotoxic CD4+ T cells in association with frequently expressed HLA class II molecules supports the application of this antigen to either specific diagnosis or subunit vaccine design.

L24 ANSWER 3 OF 5 MEDLINE

AN 2002468524 MEDLINE

DN 22215675 PubMed ID: 12228269

TI Epitope mapping of the immunodominant antigen TB10.4 and the two homologous proteins TB10.3 and TB12.9, which constitute a subfamily of the esat-6 gene family.

AU Skjot Rikke Louise Vinther; Brock Inger; Arend Sandra M; Munk Martin E; Theisen Michael; Ottenhoff Tom H M; Andersen Peter

CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.

SO INFECTION AND IMMUNITY, (2002 Oct) 70 (10) 5446-53. Journal code: 0246127. ISSN: 0019-9567.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200210

ED Entered STN: 20020914
Last Updated on STN: 20021019
Entered Medline: 20021018

AB The human **T-cell recognition** of the low-molecular-mass culture filtrate antigen TB10.4 was evaluated in

detail. The molecule was strongly recognized by T cells isolated from **tuberculosis** (TB) patients and from BCG-vaccinated donors. The epitopes on TB10.4 were mapped with overlapping peptides and found to be distributed throughout the molecule. The broadest response was found in TB patients, whereas the response in BCG-vaccinated donors was focused mainly toward a dominant epitope located in the N terminus (amino acids 1 to 18). The gene encoding TB10.4 was found to belong to a subfamily within the esat-6 family that consists of the three highly homologous proteins TB10.4, TB10.3, and TB12.9 (Rv0288, Rv3019c, and Rv3017c, respectively). Southern blot analysis combined with database searches revealed that the three members of the TB10.4 family were present only in strains of the Mycobacterium **tuberculosis** complex, including BCG, and M. kansasii, whereas other atypical mycobacteria had either one (M. avium, M. intracellulare, and M. marinum) or none (M. scrofulaceum, M. fortuitum, and M. szulgai) of the genes. The fine specificity of the T-cell response to the three closely related esat-6 family members was markedly different, with only a few epitopes shared between the molecules. Minimal differences in the amino acid sequence translated into large differences in recognition by T cells and secretion of gamma interferon. In general, the peptides from TB10.4 stimulated the largest responses, but epitopes unique to both TB10.3 and TB12.9 were found. The relevance of the findings for TB vaccine development and as a potential mechanism for immune evasion is discussed.

L24 ANSWER 4 OF 5 MEDLINE
AN 2000417674 MEDLINE
DN 20336480 PubMed ID: 10875783
TI Multiple epitopes from the Mycobacterium **tuberculosis** ESAT-6 antigen are **recognized** by antigen-specific human **T cell** lines.
AU Mustafa A S; Oftung F; Amoudy H A; Madi N M; Abal A T; Shaban F; Rosen Krands I; Andersen P
CS Department of Microbiology, Kuwait University, Safat 13110, Kuwait.. abusalim@hsc.kuniv.edu.kw
SO CLINICAL INFECTIOUS DISEASES, (2000 Jun) 30 Suppl 3 S201-5. Journal code: 9203213. ISSN: 1058-4838.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200009
ED Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000906
AB A synthetic-peptide approach was used to map epitope regions of the Mycobacterium **tuberculosis** 6-kDa early secreted antigen target (ESAT-6) by testing human CD4(+) T cell lines for secretion of IFN-gamma in response to recombinant ESAT-6 (rESAT-6) and overlapping 20-mer peptides covering the antigen sequence. The results demonstrate that all of the ESAT-6 peptides screened were able to induce IFN-gamma secretion from one or more of the T cell lines tested. Some of the individual T cell lines showed the capacity to respond to all peptides. Human leukocyte antigen (HLA-DR) typing of the donors showed that rESAT-6 was presented to T cells in association with multiple HLA-DR molecules. The results suggest that frequent recognition of the M. **tuberculosis** ESAT-6 antigen by T cells from patients with **tuberculosis** is due to the presence of multiple epitopes scattered throughout the ESAT-6 sequence.

L24 ANSWER 5 OF 5 MEDLINE DUPLICATE 1
AN 1998114377 MEDLINE
DN 98114377 PubMed ID: 9453632
TI B-cell epitopes and quantification of the ESAT-6 protein of Mycobacterium

tuberculosis.
AU Harboe M; Malin A S; Dockrell H S; Wiker H G; Ulvund G; Holm A; Jorgensen M C; Andersen P
CS Institute of Immunology and Rheumatology, University of Oslo, Norway.. morten.harboe@labmed.uio.no
SO INFECTION AND IMMUNITY, (1998 Feb) 66 (2) 717-23.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980212
AB ESAT-6 is an important **T-cell** antigen **recognized** by protective T cells in animal models of infection with Mycobacterium **tuberculosis**. In an enzyme-linked immunosorbent assay (ELISA) with overlapping peptides spanning the sequence of ESAT-6, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the molecule. Assays with synthetic truncated peptides allowed a precise mapping of the epitope to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two additional areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native ESAT-6. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The assay was suitable for quantification of ESAT-6 in M. **tuberculosis** antigen preparations, showing no reactivity with M. bovis BCG Tokyo culture fluid, used as a negative control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of ESAT-6 expression from vaccinia virus constructs containing the esat-6 gene; this expression could not be identified by standard immunoblotting.

=> s l20 and peptid? (5a) mapping
L25 3 L20 AND PEPTID? (5A) MAPPING

=> d bib ab 1-3

L25 ANSWER 1 OF 3 MEDLINE
AN 1998114377 MEDLINE
DN 98114377 PubMed ID: 9453632
TI B-cell epitopes and quantification of the ESAT-6 protein of Mycobacterium **tuberculosis**.
AU Harboe M; Malin A S; Dockrell H S; Wiker H G; Ulvund G; Holm A; Jorgensen M C; Andersen P
CS Institute of Immunology and Rheumatology, University of Oslo, Norway.. morten.harboe@labmed.uio.no
SO INFECTION AND IMMUNITY, (1998 Feb) 66 (2) 717-23.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980224

SO Comparative and Functional Genomics, (December 2002, 2002) Vol. 3, No. 6, pp. 470-483. print.
ISSN: 1531-6912.
DT Article
LA English
AB The plasma membrane of Mycobacterium tuberculosis is likely to contain proteins that could serve as novel drug targets, diagnostic probes or even components of a vaccine against tuberculosis. With this in mind, we have undertaken proteome analysis of the membrane of M. tuberculosis H37Rv. Isolated membrane vesicles were extracted with either a detergent (Triton X114) or an alkaline buffer (carbonate) following two of the protocols recommended for membrane protein enrichment. Proteins were resolved by 2D-GE using immobilized pH gradient (IPG) strips, and identified by **peptide** mass **mapping** utilizing the M. tuberculosis genome database. The two extraction procedures yielded patterns with minimal overlap. Only two proteins, both HSPs, showed a common presence. MALDI-MS analysis of 61 spots led to the identification of 32 proteins, 17 of which were new to the M. tuberculosis proteome database. We classified 19 of the identified proteins as 'membrane-associated'; 14 of these were further classified as 'membrane-bound', three of which were lipoproteins. The remaining proteins included four heat-shock proteins and several enzymes involved in energy or lipid metabolism. Extraction with Triton X114 was found to be more effective than carbonate for detecting 'putative' M. tuberculosis membrane proteins. The protocol was also found to be suitable for comparing BCG and M. tuberculosis membranes, identifying **ESAT-6** as being expressed selectively in M. tuberculosis. While this study demonstrates for the first time some of the membrane proteins of M. tuberculosis, it also underscores the problems associated with proteomic analysis of a complex membrane such as that of a mycobacterium.

L27 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 1998:120633 BIOSIS
DN PREV199800120633
TI B-cell epitopes and quantification of the **ESAT-6** protein of Mycobacterium tuberculosis.
AU Harboe, Morten (1); Malin, Adam S.; Dockrell, Hazel S.; Wiker, Harald Gotten; Ulvund, Gunni; Holm, Arne; Jorgensen, Mikala Clok; Andersen, Peter
CS (1) Inst. Immunol. Rheumatol., Univ. Oslo, Fr. Qvams gate 1, N-0172 Oslo Norway
SO Infection and Immunity, (Feb., 1998) Vol. 66, No. 2, pp. 717-723.
ISSN: 0019-9567.
DT Article
LA English
AB **ESAT-6** is an important T-cell antigen recognized by protective T cells in animal models of infection with Mycobacterium tuberculosis. In an enzyme-linked immunosorbent assay (ELISA) with overlapping peptides spanning the sequence of **ESAT-6**, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the molecule. Assays with synthetic truncated **peptides** allowed a precise **mapping** of the epitope to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two additional areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native **ESAT-6**. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The assay was suitable for quantification of **ESAT-6** in M. tuberculosis antigen preparations, showing no reactivity with M. bovis BCG Tokyo

culture fluid, used as a negative control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of **ESAT-6** expression from vaccinia virus constructs containing the **esat-6** gene; this expression could not be identified by standard immunoblotting.

L27 ANSWER 3 OF 3 MEDLINE
AN 97025462 MEDLINE
DN 97025462 PubMed ID: 8871652
TI Key epitopes on the **ESAT-6** antigen recognized in mice during the recall of protective immunity to Mycobacterium tuberculosis.
AU Brandt L; Oettinger T; Holm A; Andersen A B; Andersen P
CS The TB Research Unit, Bacterial Vaccine Department, Statens Seruminstitut, Copenhagen, Denmark.
SO JOURNAL OF IMMUNOLOGY, (1996 Oct 15) 157 (8) 3527-33.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961217
AB The recall of long-lived immunity in a mouse model of tuberculosis (TB) is defined as an accelerated accumulation of reactive T cells in the target organs. We have recently identified Ag 85B and a 6-kilodalton early secretory antigenic target, designated **ESAT-6**, as key antigenic targets recognized by these cells. In the present study, preferential recognition of the **ESAT-6** Ag during the recall of immunity was found to be shared by five of six genetically different strains of mice. Overlapping peptides spanning the sequence of **ESAT-6** were used to map two T cell epitopes on this molecule. One epitope recognized in the context of H-2b,d was located in the N-terminal part of the molecule, whereas an epitope recognized in the context of H-2a,k covered amino acids 51 to 60. Shorter versions of the N-terminal epitope allowed the precise definition of a 13-amino acid core sequence recognized in the context of H-2b. The peptide covering the N-terminal epitope was immunogenic, and a T cell response with the same fine specificity as that induced during TB infection was generated by immunization with the peptide in IFA. In the C57BL/6j strain, this single epitope was recognized by an exceedingly high frequency of splenic T cells (approximately 1:1000), representing 25 to 35% of the total culture filtrate-reactive T cells recruited to the site of infection during the first phase of the recall response. These findings emphasize the relevance of this Ag in the immune response to TB and suggest that immunologic recognition in the first phase of infection is a highly restricted event dominated by a limited number of T cell clones.

=> s tuberculosis and peptide (5a) ampping
L28 0 TUBERCULOSIS AND PEPTIDE (5A) AMPPING

=> s tuberculosis and peptide (5a) mapping
L29 52 TUBERCULOSIS AND PEPTIDE (5A) MAPPING

=> dup rem l29
PROCESSING COMPLETED FOR L29
L30 30 DUP REM L29 (22 DUPLICATES REMOVED)

=> d bib ab 1-30

L30 ANSWER 1 OF 30 EMBASE

AN 2003133614 EMBASE
TI Synthetic peptides identify promiscuous human Th1 cell epitopes of the secreted mycobacterial antigen MPB70.
AU Al-Attiyah R.; Shaban F.A.; Wiker H.G.; Oftung F.; Mustafa A.S.
CS R. Al-Attiyah, Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait. rj-alattiyah@hsc.kuniv.edu.kw
SO Infection and Immunity, (1 Apr 2003) 71/4 (1953-1960).
Refs: 60
ISSN: 0019-9567 CODEN: INFIBR
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB MPB70 is a secreted protein of Mycobacterium bovis and Mycobacterium **tuberculosis** which stimulates both cellular and humoral immune responses during infection with bovine and human tubercle bacilli. In addition, vaccination with MPB70 has been shown to induce Th1 cell responses and protection in animal models of **tuberculosis**. The present study was carried out to map the dominant human Th1 cell epitopes of MPB70 in relation to major histocompatibility complex (MHC) class II restriction in healthy subjects showing strong T-cell responses to complex mycobacterial antigens. Peripheral blood mononuclear cells (PBMC) from HLA-DR-typed donors were tested with complex mycobacterial antigens (whole-cell M. **tuberculosis** and M. **tuberculosis** culture filtrates), with MPB70 purified from the culture filtrate of M. bovis BCG Tokyo, and with 13 synthetic peptides (25-mers overlapping by 10 residues) covering the sequence of MPB70. The donors that responded to the complex antigens and MPB70 also responded to the cocktail of synthetic MPB70 peptides. Testing of PBMC with individual peptides showed that peptides p5 (amino acids [aa] 61 to 85), p6 (aa 76 to 100), p8 (aa 106 to 130), p12 (aa 166 to 190), and p13 (aa 181 to 193) were most frequently recognized in proliferation and gamma interferon (IFN-.gamma.) assays. Testing of antigen-specific CD4(+) T-cell lines with the individual peptides of MPB70 confirmed that peptides p8, p12, and p13 contain immunodominant Th1 cell epitopes of MPB70. MHC restriction analysis with HLA-typed donors showed that MPB70 and its immunodominant peptides were presented to T cells promiscuously. The T-cell lines responding to MPB70 and peptides p8, p12, and p13 in IFN-.gamma. assays mediated antigen-peptide-specific cytotoxic activity against monocytes/macrophages pulsed with the whole-protein antigen or the peptides. In conclusion, the promiscuous recognition of MPB70 and its immunodominant peptide defined epitopes (aa 106 to 130 and 166 to 193) by IFN-.gamma.-producing Th1 cells supports possible application of this secreted antigen to subunit vaccine design.

L30 ANSWER 2 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2003:103124 BIOSIS
DN PREV200300103124
TI Proteome analysis of the plasma membrane of Mycobacterium **tuberculosis**.
AU Sinha, Sudhir (1); Arora, Shalini; Kosalai, K.; Namane, Abdelkader; Pym, Alex S.; Cole, Stewart T.
CS (1) Division of Biochemistry, Central Drug Research Institute, PO Box 173, Lucknow, 226001, India: sinhas@lycos.com India
SO Comparative and Functional Genomics, (December 2002, 2002) Vol. 3, No. 6, pp. 470-483. print.
ISSN: 1531-6912.

DT Article
LA English
AB The plasma membrane of Mycobacterium **tuberculosis** is likely to contain proteins that could serve as novel drug targets, diagnostic probes or even components of a vaccine against **tuberculosis**. With this in mind, we have undertaken proteome analysis of the membrane of M. **tuberculosis** H37Rv. Isolated membrane vesicles were extracted with either a detergent (Triton X114) or an alkaline buffer (carbonate) following two of the protocols recommended for membrane protein enrichment. Proteins were resolved by 2D-GE using immobilized pH gradient (IPG) strips, and identified by **peptide** mass **mapping** utilizing the M. **tuberculosis** genome database. The two extraction procedures yielded patterns with minimal overlap. Only two proteins, both HSPs, showed a common presence. MALDI-MS analysis of 61 spots led to the identification of 32 proteins, 17 of which were new to the M. **tuberculosis** proteome database. We classified 19 of the identified proteins as 'membrane-associated'; 14 of these were further classified as 'membrane-bound', three of which were lipoproteins. The remaining proteins included four heat-shock proteins and several enzymes involved in energy or lipid metabolism. Extraction with Triton X114 was found to be more effective than carbonate for detecting 'putative' M. **tuberculosis** membrane proteins. The protocol was also found to be suitable for comparing BCG and M. **tuberculosis** membranes, identifying ESAT-6 as being expressed selectively in M. **tuberculosis**. While this study demonstrates for the first time some of the membrane proteins of M. **tuberculosis**, it also underscores the problems associated with proteomic analysis of a complex membrane such as that of a mycobacterium.

L30 ANSWER 3 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 2001:731001 CAPLUS
DN 135:284066
TI Nucleic acids and proteins associated with human prostate cancer and their uses in therapy and diagnosis
IN Xu, Jiangchun; Dillon, Davin C.; Mitcham, Jennifer L.; Harlocker, Susan L.; Jiang, Yuqiu; Kalos, Michael D.; Fanger, Gary Richard; Retter, Marc W.; Stolk, John A.; Day, Craig H.; Vedvick, Thomas S.; Carter, Darrick; Li, Samuel X.; Wang, Aijun; Skeiky, Yasir A. W.; Hepler, William T.; Henderson, Robert A.
PA Corixa Corporation, USA
SO PCT Int. Appl., 579 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 23

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001073032	A2	20011004	WO 2001-US9919	20010327
	WO 2001073032	A3	20030313		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6512094	B1	20030128	US 2000-593793	20000613
	EP 1311673	A2	20030521	EP 2001-922786	20010327
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI	US 2000-536857	A	20000327		

US 2000-568100 A 20000509
US 2000-570737 A 20000512
US 2000-593793 A 20000613
US 2000-605783 A 20000627
US 2000-636215 A 20000810
US 2000-651236 A 20000829
US 2000-657279 A 20000906
US 2000-679426 A 20001002
US 2000-685166 A 20001010
US 2000-709729 A 20001109
WO 2001-US9919 W 20010327

AB Compns. and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compns. comprise one or more prostate-specific polypeptides, immunogenic portions thereof, and polynucleotides that encode such polypeptides as identified by PCR-based cDNA library subtraction. Chromosomal mapping, tissue expression profiling, and prepn. of fusion proteins (esp. with the Ra12 portion of the Mycobacterium **tuberculosis** serine protease MTB32A) are carried out. Epitope mapping is carried out on some of the polypeptides (e.g., P501S) to identify immunogenic peptides. Antigen-presenting cells that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides are also provided. The disclosed compns. are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

L30 ANSWER 4 OF 30 MEDLINE
AN 2001545066 MEDLINE
DN 21145799 PubMed ID: 11248033
TI Crystal structure of cytochrome P450 14alpha -sterol demethylase (CYP51) from Mycobacterium **tuberculosis** in complex with azole inhibitors.
AU Podust L M; Poulos T L; Waterman M R
CS Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146, USA.. podustlm@ctrvax.vanderbilt.edu
NC CA68485 (NCI)
DK20593 (NIDDK)
ES00267 (NIEHS)
GM33688 (NIGMS)
GM37942 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Mar 13) 98 (6) 3068-73.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1E9X; PDB-1EA1
EM 200112
ED Entered STN: 20011011
Last Updated on STN: 20020121
Entered Medline: 20011204
AB Cytochrome P450 14alpha-sterol demethylases (CYP51) are essential enzymes in sterol biosynthesis in eukaryotes. CYP51 removes the 14alpha-methyl group from sterol precursors such as lanosterol, obtusifoliol, dihydrolanosterol, and 24(28)-methylene-24,25-dihydrolanosterol. Inhibitors of CYP51 include triazole antifungal agents fluconazole and itraconazole, drugs used in treatment of topical and systemic mycoses. The 2.1- and 2.2-A crystal structures reported here for 4-phenylimidazole- and fluconazole-bound CYP51 from Mycobacterium **tuberculosis** (MTCYP51) are the first structures of an authentic P450 drug target. MTCYP51 exhibits the P450 fold with the exception of two striking differences-a bent I helix and an open conformation of BC loop-that define an active site-access channel running along the heme plane perpendicular

to the direction observed for the substrate entry in P450BM3. Although a channel analogous to that in P450BM3 is evident also in MTCYP51, it is not open at the surface. The presence of two different channels, with one being open to the surface, suggests the possibility of conformationally regulated substrate-in/product-out openings in CYP51. Mapping mutations identified in Candida albicans azole-resistant isolates indicates that azole resistance in fungi develops in protein regions involved in orchestrating passage of CYP51 through different conformational stages along the catalytic cycle rather than in residues directly contacting fluconazole. These new structures provide a basis for rational design of new, more efficacious antifungal agents as well as insight into the molecular mechanism of P450 catalysis.

L30 ANSWER 5 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:378769 BIOSIS
DN PREV200100378769
TI Identification of acidic, low molecular mass proteins of Mycobacterium **tuberculosis** strain H37Rv by matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry.
AU Mattow, Jens (1); Jungblut, Peter R.; Mueller, Eva-Christina; Kaufmann, Stefan H. E.
CS (1) Department of Immunology, Max-Planck-Institute for Infection Biology, Schumannstr. 21/22, D-10117, Berlin: mattow@mpiib-berlin.mpg.de Germany
SO Proteomics, (April, 2001) Vol. 1, No. 4, pp. 494-507. print.
ISSN: 1615-9853.
DT Article
LA English
SL English
AB Matrix-assisted laser desorption/ionization-mass spectrometry **peptide** mass **mapping** and nano-electrospray ionization tandem mass spectrometry were used to identify acidic, low molecular mass proteins of Mycobacterium **tuberculosis** strain H37Rv. Proteins were extracted from whole cell lysates of mycobacteria, separated by high resolution two-dimensional electrophoresis (2-DE) and analysed by mass spectrometry (MS). Silver-stained 2-DE patterns resolved about 1800 distinct protein species, 190 of which had an observed isoelectric point and molecular mass in the range of pH 4 to 6 and 6 to 15 kDa, respectively. Seventy-six spots from this range were excised from Coomassie Brilliant Blue G250-stained gels and analysed by MS, from which 72 were identified. These spots were shown to represent products of as many as 50 different protein-coding genes. Ten genes gave rise to more than one protein species. Eleven spots contained more than one protein. The present study led to the identification of 15 mycobacterial proteins with assigned putative functions, 28 conserved hypothetical proteins and one unknown protein. Most proteins of the latter two groups had previously been predicted at the DNA level only. Six additional spots were shown to comprise proteins encoded by open reading frames that have not been predicted for M. **tuberculosis** H37Rv by genomic investigations.

L30 ANSWER 6 OF 30 MEDLINE
AN 2001076846 MEDLINE
DN 20540106 PubMed ID: 11086086
TI Identification of major epitopes of Mycobacterium **tuberculosis** AG85B that are recognized by HLA-A*0201-restricted CD8+ T cells in HLA-transgenic mice and humans.
AU Geluk A; van Meijgaarden K E; Franken K L; Drijfhout J W; D'Souza S; Necker A; Huygen K; Ottenhoff T H
CS Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.. ageluk@lumc.nl
SO JOURNAL OF IMMUNOLOGY, (2000 Dec 1) 165 (11) 6463-71.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20030111
Entered Medline: 20010111
AB CD8(+) T cells are thought to play an important role in protective immunity to **tuberculosis**. Although several nonprotein ligands have been identified for CD1-restricted CD8(+) CTLs, epitopes for classical MHC class I-restricted CD8(+) T cells, which most likely represent a majority among CD8(+) T cells, have remained ill defined. HLA-A*0201 is one of the most prevalent class I alleles, with a frequency of over 30% in most populations. HLA-A2/K(b) transgenic mice were shown to provide a powerful model for studying induction of HLA-A*0201-restricted immune responses in vivo. The Ag85 complex, a major component of secreted Mycobacterium **tuberculosis** proteins, induces strong CD4(+) T cell responses in M. **tuberculosis**-infected individuals, and protection against **tuberculosis** in Ag85-DNA-immunized animals. In this study, we demonstrate the presence of HLA class I-restricted, CD8(+) T cells against Ag85B of M. **tuberculosis** in HLA-A2/K(b) transgenic mice and HLA-A*0201(+) humans. Moreover, two immunodominant Ag85 peptide epitopes for HLA-A*0201-restricted, M. **tuberculosis**-reactive CD8(+) CTLs were identified. These CD8(+) T cells produced IFN-gamma and TNF-alpha and recognized Ag-pulsed or bacillus Calmette-Guerin-infected, HLA-A*0201-positive, but not HLA-A*0201-negative or uninfected human macrophages. This CTL-mediated killing was blocked by anti-CD8 or anti-HLA class I mAb. Using fluorescent peptide/HLA-A*0201 tetramers, Ag85-specific CD8(+) T cells could be visualized in bacillus Calmette-Guerin-responsive, HLA-A*0201(+) individuals. Collectively, our results demonstrate the presence of HLA class I-restricted CD8(+) CTL against a major Ag of M. **tuberculosis** and identify Ag85B epitopes that are strongly recognized by HLA-A*0201-restricted CD8(+) T cells in humans and mice. These epitopes thus represent potential subunit components for the design of vaccines against **tuberculosis**.

L30 ANSWER 7 OF 30 MEDLINE DUPLICATE 3
AN 2000229267 MEDLINE
DN 20229267 PubMed ID: 10768780
TI Mapping and identification of Mycobacterium **tuberculosis** proteins by two-dimensional gel electrophoresis, microsequencing and immunodetection.
AU Rosenkrands I; Weldingh K; Jacobsen S; Hansen C V; Florio W; Gianetri I; Andersen P
CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.
SO ELECTROPHORESIS, (2000 Mar) 21 (5) 935-48.
Journal code: 8204476. ISSN: 0173-0835.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000606
AB Mycobacterium **tuberculosis** is the infectious agent giving rise to human **tuberculosis**. The entire genome of M. **tuberculosis**, comprising approximately 4000 open reading frames, has been sequenced. The huge amount of information released from this project has facilitated proteome analysis of M. **tuberculosis**. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was applied to fractions derived from M. **tuberculosis** culture filtrate, cell

wall, and cytosol, resulting in the resolution of 376, 413, and 395 spots, respectively, in silver-stained gels. By microsequencing and immunodetection, 38 culture filtrate proteins were identified and mapped, of which 12 were identified for the first time. In the same manner, 23 cell wall proteins and 19 cytosol proteins were identified and mapped, with 9 and 10, respectively, being novel proteins. One of the novel proteins was not predicted in the genome project, and for four of the identified proteins alternative start codons were suggested. Fourteen of the culture filtrate proteins were proposed to possess signal sequences. Seven of these proteins were microsequenced and the N-terminal sequences obtained confirmed the prediction. The data presented here are an important complement to the genetic information, and the established 2-D PAGE maps (also available at: www.ssi.dk/publichealth/tbimmun) provide a basis for comparative studies of protein expression.

L30 ANSWER 8 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2000:267148 BIOSIS
DN PREV200000267148
TI Immunoreactivity of peptides generated by limited proteolysis of 71-kDa cell wall protein of Mycobacterium **tuberculosis** H37Ra using PLG-microparticles.
AU Dhiman, N.; Khuller, G. K. (1)
CS (1) Biochemistry Department, PGIMER, Chandigarh, 160012 India
SO Letters in Applied Microbiology, (May, 2000) Vol. 30, No. 5, pp. 345-350. print..
ISSN: 0266-8254.
DT Article
LA English
SL English
AB **Peptide mapping** by limited proteolysis of a highly protective 71-kDa cell wall-associated protein of Mycobacterium **tuberculosis** H37Ra was carried out in order to identify key protective determinants within the native protein. The 71-kDa protein, which had an isoelectric point of 4.25, was digested into eight major bands at 48 h using trypsin and pepsin at equal enzyme to protein ratios (pH 5.5). The in vitro lymphocyte reactivity of individual peptides suggested P1, P2 and P5 to be significantly immunoreactive in mice immunized with native 71-kDa-polylactide-coglyeolide (PLG); however, the reactivity was significantly lower than that of the native 71-kDa protein. Immunization of mice with a pooled fraction (upper fraction-71 kDa) of more immunoreactive peptides (consisting of P1 and P2) did not further boost their immunoreactivity. However, P1 and P2 exhibited comparable or even higher lymphocyte proliferation in human tuberculous and control subjects. These data suggest distinct antigenic specificities in humans and mice and further substantiate the use of the 71-kDa protein or its peptides P1 and P2 as potential vaccine candidates for **tuberculosis**.

L30 ANSWER 9 OF 30 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2000118699 EMBASE
TI Cross-reactive epitopes and HLA-restriction elements in human T cell recognition of the Mycobacterium leprae 18-kD heat shock protein.
AU Mustafa A.S.; Lundin K.E.A.; Meloen R.H.; Oftung F.
CS Prof. A.S. Mustafa, Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait. abusalim@hsc.kuniv.edu.kw
SO Clinical and Experimental Immunology, (2000) 120/1 (85-92).
Refs: 30
ISSN: 0009-9104 CODEN: CEXIAL
CY United Kingdom
DT Journal; Article
FS 026 Immunology, Serology and Transplantation

LA English
SL English
AB We have previously demonstrated that the Mycobacterium leprae 18-kD heat shock protein (HSP18) is represented among the antigenic targets of human T cell responses induced by M. leprae immunization and that the peptide 38-50 serves as an immunodominant epitope recognized by CD4+ T cell clones By using peripheral blood mononuclear cells and T cell lines from the same donor group, we have in this study shown that the M. leprae HSP18 and peptide 38-50 were recognized by memory T cells 8 years after immunization with M. leprae. The finding that M. boris BCG-induced T cell lines responded to M. leprae HSP18, but not to the peptide 38-50, suggested the existence of additional T cell epitopes of a cross-reactive nature. Consistent with this, testing of the T cell lines for proliferative responses to the complete HSP18 molecule, truncated HSP18 (amino acid (aa) residues 38-148) and overlapping synthetic peptides, made it possible to identify two cross-reactive epitope regions defined by aa residues 1-38 and 41-55. While peptide 38-50-reactive T cell clones showed limited cross-reactivity by responding to M. leprae, M. avium and M. scrofulaceum, the T cell lines specific to the epitopes 1-38 and 41-55 were broadly cross-reactive, as demonstrated by their response to M. leprae, M. **tuberculosis** complex, M. avium and other mycobacteria. MHC restriction analysis of the HSP18-responding T cell lines showed that the epitopes 1-38 and 38-50 were presented by one of the two HLA-DR molecules expressed from self HLA-DRB1 genes, whereas the epitope 41-55 was recognized in the presence of autologous as well as HLA-DR and HLA-DQ mismatched allogeneic antigen- presenting cells. The results obtained in this study made it possible to identify cross-reactive T cell epitopes of the M. leprae HSP18, and provide an explanation for T cell recognition of this antigen in individuals infected with species of the M. **tuberculosis** complex or environmental mycobacteria.

L30 ANSWER 10 OF 30 MEDLINE
AN 1999446338 MEDLINE
DN 99446338 PubMed ID: 10517141
TI The dominance of arginine-containing peptides in MALDI-derived tryptic mass fingerprints of proteins.
AU Krause E; Wenschuh H; Jungblut P R
CS Institute of Molecular Pharmacology, Berlin, Germany.
SO ANALYTICAL CHEMISTRY, (1999 Oct 1) 71 (19) 4160-5.
Journal code: 0370536. ISSN: 0003-2700.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000209
Last Updated on STN: 20000209
Entered Medline: 20000201
AB Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful tool for mass finger-printing of peptide mixtures obtained after enzymatic ingel digestion of proteins separated by two-dimensional electrophoresis (2-DE). In the course of a proteome analysis of mycobacteria using mass spectrometric identification, it was found that 94% of the most intense MALDI-MS peaks denote peptides bearing arginine at the C-terminal end. The effect was demonstrated to be equally prominent using an equimolar mixture of the synthetic peptides known to be present in the tryptic digest of the mycobacterial 35 kDa antigen ("synthetic mass map"). In addition, several binary mixtures of synthetic peptides differing exclusively at the C terminus (Arg or Lys) were examined to rationalize the higher sensitivity toward arginine-containing peptides. The extent of the effect described depends on the matrix used and may facilitate a more reliable assignment of mass fingerprint data to protein sequences in databases.

L30 ANSWER 11 OF 30 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 1999152562 EMBASE
TI T-cell epitope mapping of the three most abundant extracellular proteins of Mycobacterium **tuberculosis** in outbred guinea pigs.
AU Lee B.-Y.; Horwitz M.A.
CS M.A. Horwitz, Department of Medicine, CHS 37-121, UCLA School of Medicine, 10833 Le Conte Ave., Los Angeles, CA 90095-1688, United States. MHorwitz@med1.medsch.ucla.edu
SO Infection and Immunity, (1999) 67/5 (2665-2670).
Refs: 20
ISSN: 0019-9567 CODEN: INFIBR
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB The three most abundant extracellular proteins of Mycobacterium **tuberculosis**, the 30-, 32-, and 16-kDa major extracellular proteins, are particularly promising vaccine candidates. We have mapped T-cell epitopes of these three proteins in outbred guinea pigs by immunizing the animals with each protein and assaying splenic lymphocyte proliferation against a series of overlapping synthetic peptides covering the entire length of the mature proteins. The 30-kDa protein contained nine immunodominant epitopes, the 32- kDa protein contained two immunodominant epitopes, and the 16-kDa protein contained a highly immunodominant region at its N terminus. The immunodominant epitopes of the 30- and 32-kDa proteins in outbred guinea pigs were frequently identified in healthy purified-protein-derivative-positive or BCG-vaccinated individuals in previous studies. The immunodominant epitopes of these major extracellular proteins have potential utility in an epitope- based vaccine against **tuberculosis**.

L30 ANSWER 12 OF 30 MEDLINE
AN 97443981 MEDLINE
DN 97443981 PubMed ID: 9298652
TI 'Proteomic contigs' of Mycobacterium **tuberculosis** and Mycobacterium bovis (BCG) using novel immobilised pH gradients.
AU Urquhart B L; Atsalos T E; Roach D; Basseal D J; Bjellqvist B; Britton W L; Humphery-Smith I
CS Centre for Proteome Research and Gene-Product Mapping, National Innovation Centre, Eveleigh, Australia.
SO ELECTROPHORESIS, (1997 Aug) 18 (8) 1384-92.
Journal code: 8204476. ISSN: 0173-0835.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199710
ED Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023
AB **Tuberculosis** remains a major health problem throughout the world and the failure of the existing bacille Calmette-Guerin (BCG) vaccine in recent trials has prompted a search for potential replacements. Recent advances in molecular and cell biology have cast doubts on the ability of genetic analysis alone to predict polygenic human diseases and other complex phenotypes and have therefore redirected our attention to proteome studies to complement information obtained from DNA sequencing initiatives. Novel acidic (pH 2.3-5) and basic (pH 6-11) IPG gel gradients were employed in conjunction with commercially available pH 4-7

gradients to significantly increase (fourfold) the number of protein spots previously resolved on two-dimensional (2-D) gels of Mycobacterium species. A total of 772 and 638 protein spots were observed for M. bovis BCG and M. **tuberculosis** H37Rv, respectively, the latter corresponding to only the pH regions 4-7 and 6-11. Of interest was the bimodal distribution observed for proteins separated from M. bovis BCG across both M(r) and pH ranges. Some differences in protein expression were observed between these two organisms, contrary to what may have been expected considering the high degree of conservation in gene order and sequence similarity between homologous genes. Further work will be directed towards a more detailed analysis of these differences, so as to allow more accurate diagnosis between vaccination and active **tuberculosis**. The latter is of major importance to epidemiological studies and for patient management.

L30 ANSWER 13 OF 30 MEDLINE
AN 97477407 MEDLINE
DN 97477407 PubMed ID: 9334363
TI Mycobacterium **tuberculosis** chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease.
AU Meghji S; White P A; Nair S P; Reddi K; Heron K; Henderson B; Zaliani A; Fossati G; Mascagni P; Hunt J F; Roberts M M; Coates A R
CS Maxillofacial Surgery Research Unit, Eastman Dental Institute, University College London, United Kingdom.
SO JOURNAL OF EXPERIMENTAL MEDICINE, (1997 Oct 20) 186 (8) 1241-6.
Journal code: 2985109R. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199711
ED Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971121
AB Pott's disease (spinal **tuberculosis**), a condition characterized by massive resorption of the spinal vertebrae, is one of the most striking pathologies resulting from local infection with Mycobacterium **tuberculosis** (Mt; Boachie-Adjei, O., and R.G. Squillante. 1996. Orthop. Clin. North Am. 27:95-103). The pathogenesis of Pott's disease is not established. Here we report for the first time that a protein, identified by a monoclonal antibody to be the Mt heat shock protein (Baird, P.N., L.M. Hall, and A.R.M. Coates. 1989. J. Gen. Microbiol. 135:931-939) chaperonin (cpn) 10, is responsible for the osteolytic activity of this bacterium. Recombinant Mt cpn10 is a potent stimulator of bone resorption in bone explant cultures and induces osteoclast recruitment, while inhibiting the proliferation of an osteoblast bone-forming cell line. Furthermore, we have found that synthetic peptides corresponding to sequences within the flexible loop and sequence 65-70 of Mt cpn10 may comprise a single conformational unit which encompasses its potent bone-resorbing activity. Our findings suggest that Mt cpn10 may be a valuable pharmacological target for the clinical therapy of vertebral **tuberculosis** and possibly other bone diseases.

L30 ANSWER 14 OF 30 MEDLINE
AN 97025462 MEDLINE
DN 97025462 PubMed ID: 8871652
TI Key epitopes on the ESAT-6 antigen recognized in mice during the recall of protective immunity to Mycobacterium **tuberculosis**.
AU Brandt L; Oettinger T; Holm A; Andersen A B; Andersen P
CS The TB Research Unit, Bacterial Vaccine Department, Statens Seruminstitut, Copenhagen, Denmark.
SO JOURNAL OF IMMUNOLOGY, (1996 Oct 15) 157 (8) 3527-33.
Journal code: 2985117R. ISSN: 0022-1767.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961217
AB The recall of long-lived immunity in a mouse model of **tuberculosis** (TB) is defined as an accelerated accumulation of reactive T cells in the target organs. We have recently identified Ag 85B and a 6-kilodalton early secretory antigenic target, designated ESAT-6, as key antigenic targets recognized by these cells. In the present study, preferential recognition of the ESAT-6 Ag during the recall of immunity was found to be shared by five of six genetically different strains of mice. Overlapping peptides spanning the sequence of ESAT-6 were used to map two T cell epitopes on this molecule. One epitope recognized in the context of H-2b,d was located in the N-terminal part of the molecule, whereas an epitope recognized in the context of H-2a,k covered amino acids 51 to 60. Shorter versions of the N-terminal epitope allowed the precise definition of a 13-amino acid core sequence recognized in the context of H-2b. The peptide covering the N-terminal epitope was immunogenic, and a T cell response with the same fine specificity as that induced during TB infection was generated by immunization with the peptide in IFA. In the C57BL/6j strain, this single epitope was recognized by an exceedingly high frequency of splenic T cells (approximately 1:1000), representing 25 to 35% of the total culture filtrate-reactive T cells recruited to the site of infection during the first phase of the recall response. These findings emphasize the relevance of this Ag in the immune response to TB and suggest that immunologic recognition in the first phase of infection is a highly restricted event dominated by a limited number of T cell clones.

L30 ANSWER 15 OF 30 MEDLINE
AN 95347792 MEDLINE
DN 95347792 PubMed ID: 7622204
TI Evidence for glycosylation sites on the 45-kilodalton glycoprotein of Mycobacterium **tuberculosis**.
AU Dobos K M; Swiderek K; Khoo K H; Brennan P J; Belisle J T
CS Department of Microbiology, Colorado State University, Fort Collins 80523, USA.
NC AI-25147 (NIAID)
AI-35243 (NIAID)
CA-33572 (NCI)
SO INFECTION AND IMMUNITY, (1995 Aug) 63 (8) 2846-53.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199508
ED Entered STN: 19950911
Last Updated on STN: 19950911
Entered Medline: 19950825
AB The occurrence of glycosylated proteins in Mycobacterium **tuberculosis** has been widely reported. However, unequivocal proof for the presence of true glycosylated amino acids within these proteins has not been demonstrated, and such evidence is essential because of the predominance of soluble lipoglycans and glycolipids in all mycobacterial extracts. We have confirmed the presence of several putative glycoproteins in subcellular fractions of M. **tuberculosis** by reaction with the lectin concanavalin A. One such product, with a molecular mass of 45 kDa, was purified from the culture filtrate.

CS (1) Vet. Sci. Div., Dep. Agric. Northern Ireland, Stoney Rd., Stormont, Belfast BT4 3SD UK
SO Scandinavian Journal of Immunology, (1995) Vol. 41, No. 1, pp. 85-93. ISSN: 0300-9475.
DT Article
LA English
AB Mycobacterium bovis infection in cattle continues to be a problem in several regions, partly due to inadequate diagnostic tests. The aim of this study was to use an experimental model of the natural disease to identify T-cell epitopes from the mycobacterial 38 kDa antigen as potentially specific diagnostic reagents. A panel of overlapping synthetic peptides (16-mers with a five-residue overlap) were produced from the published amino acid sequence. It was found that peripheral blood lymphocytes from at least three of four experimentally infected animals, which were considered to be in either T-h1- or T-h1/T-h2-dominated stages of anti-mycobacterial immunity, proliferated in response to five epitopes (residues 1-27, 88-107, 122-138, 243-260 and 307-328). However, in vitro production of IFN-gamma was detected only in response to epitope 122-138, indicating a role in protective immunity. The peptides were not recognized by control, uninfected animals, but all epitopes showed various degrees of recognition by animals which were field reactors to intradermal tuberculin testing. Furthermore, epitopes 1-27, 88+- 107 and 122-138 were recognized by four breeds of cattle and by animals from separate herds, suggesting genetic permissiveness in recognition which would be essential in the development of a diagnostic test.

L30 ANSWER 18 OF 30 MEDLINE
AN 94131565 MEDLINE
DN 94131565 PubMed ID: 7507889
TI Mapping of TH1 helper T-cell epitopes on major secreted mycobacterial antigen 85A in mice infected with live Mycobacterium bovis BCG.
AU Huygen K; Lozes E; Gilles B; Drowart A; Palfliet K; Jurion F; Roland I; Art M; Dufaux M; Nyabenda J; +
CS Instituut Pasteur van Brabant, Hopital Erasme (ULB), Brussels, Belgium.
SO INFECTION AND IMMUNITY, (1994 Feb) 62 (2) 363-70.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199403
ED Entered STN: 19940318
Last Updated on STN: 19960129
Entered Medline: 19940304
AB TH1 cytokine secretion was examined in response to synthetic peptides of the 85A component of the major secreted, fibronectin-binding antigen 85 complex from Mycobacterium **tuberculosis** in seven different mouse strains infected with live M. bovis BCG. Twenty-eight overlapping 20-mer peptides covering the complete mature 295-amino-acid (AA) protein were synthesized. Significant interleukin-2 (IL-2) and gamma interferon (IFN-gamma) secretion could be measured following in vitro stimulation of spleen cells with these peptides. H-2d haplotype mice reacted preferentially against the amino-terminal half of the protein, i.e., against peptide 5 (AA 41 to 60) and especially against peptide 11 (AA 101 to 120), which contained an I-Ed binding motif. H-2b haplotype mice, on the other hand, reacted against peptides from both amino- and carboxy-terminal halves of the protein, peptide 25 (AA 241 to 260) being the most potent stimulator of IL-2 and IFN-gamma production. (BALB/c x C57BL/6)F1 animals with the H-2d/b haplotype weakly recognized peptides specific for both parental lines. Finally, CBA/J (H-2k) and major histocompatibility complex class II mutant B6.C.bm12 mice, carrying a mutant I-A beta bm12 allele on an H-2b background, reacted only very weakly to the 85A peptides. Reactive T cells isolated from lungs of

BCG-infected H-2b haplotype mice recognized the same epitopes as spleen cells, especially peptide 25. These data confirm previous findings regarding the powerful IL-2 and IFN-gamma-inducing properties of antigen 85 during infection with live M. bovis BCG.

L30 ANSWER 19 OF 30 MEDLINE
AN 94103640 MEDLINE
DN 94103640 PubMed ID: 7506279
TI Identification of an antigenic domain on Mycobacterium leprae protein antigen 85B, which is specifically recognized by antibodies from patients with leprosy.
AU Filley E; Thole J E; Rook G A; Nagai S; Waters M; Drijfhout J W; Rinke de Wit T F; De Vries R R; Abou-Zeid C
CS Department of Medical Microbiology, University College London Medical School, United Kingdom.
SO JOURNAL OF INFECTIOUS DISEASES, (1994 Jan) 169 (1) 162-9.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199402
ED Entered STN: 19940218
Last Updated on STN: 19960129
Entered Medline: 19940210
AB Sixty-three overlapping 15-oligomer peptides covering the 30-kDa protein antigen 85B of Mycobacterium leprae were tested by ELISA to identify epitopes recognized by human antibodies. Serum samples from patients with lepromatous leprosy (LL) reacted mainly with peptides comprising amino acid regions (AA) 206-230, 251-280, and 291-325. Sera of patients with active **tuberculosis** who responded to the native 30-kDa antigen did not recognize these peptides. The antibody-binding specificity to the defined B cell regions was evaluated in a blind study with 71 serum samples from patients and household contacts living in Ethiopia where leprosy is endemic. The peptide of AA 256-280 was recognized by 88% of LL patients, 15% of patients with tuberculoid leprosy, and none of the contacts. These findings suggest that there are major linear B cell epitopes on the M. leprae 30-kDa protein that are recognized by lepromin-negative LL patients, whereas lepromin-positive patients respond preferentially to conformational epitopes.

L30 ANSWER 20 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1993:370538 BIOSIS
DN PREV199396056213
TI Involvement of tryptophan(s) at the active site of polyphosphate/ATP glucokinase from Mycobacterium **tuberculosis**.
AU Hsieh, Pei-Chung; Shenoy, Bhami C.; Haase, F. Carl; Jentoft, Joyce E.; Phillips, Nelson F. B. (1)
CS (1) Dep. Biochem., Case Western Reserve Univ. Sch. Med., Cleveland, OH 44106 USA
SO Biochemistry, (1993) Vol. 32, No. 24, pp. 6243-6249.
ISSN: 0006-2960.
DT Article
LA English
AB The glucokinase (EC 2.7.1.63) from Mycobacterium **tuberculosis** catalyzes the phosphorylation of glucose using inorganic polyphosphate (poly(P)) or ATP as the phosphoryl donor. The nature of the poly(P) and ATP sites was investigated by using N-bromosuccinimide (NBS) as a probe for the involvement of tryptophan in substrate binding and/or catalysis. NBS oxidation of the tryptophan(s) resulted in fluorescence quenching with concomitant loss of both the poly(P)- and ATP-dependent glucokinase activities. The inactivation by NBS was not due to extensive structural

changes, as evidenced by similar circular dichroism spectra and fluorescence emission maxima for the native and NBS-inactivated enzyme. Both phosphoryl donor substrates in the presence of xylose afforded apprx 65% protection against inactivation by NBS. The K-m values of poly(P) and ATP were not altered due to the modification by NBS, while the catalytic efficiency of the enzyme was decreased, suggesting that the essential tryptophan(s) are involved in the catalysis of the substrates. Acrylamide quenching studies indicated that the tryptophan residue(s) were partially shielded by the substrates against quenching. The Stern-Volmer quenching constant (K-SV) of the tryptophans in unliganded glucokinase was 3.55 M-1, while K-SV values of 2.48 and 2.57 M-1 were obtained in the presence of xylose+poly(P)-5 and xylose+ATP, respectively. When the tryptophan-containing peptides were analyzed by **peptide mapping**, the same **peptide** was found to be protected by xylose+poly(P)-5 and xylose+ATP against oxidation by NBS. The two protected peptides were determined to be identical by N-terminal sequence analysis and amino acid composition. It is proposed from these results that one or both of the tryptophans present in the protected peptide may be located at a common catalytic center and that this peptide may constitute part of the poly(P) and ATP binding regions.

L30 ANSWER 21 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1993:370539 BIOSIS
DN PREV199396056214
TI Stabilization of Escherichia coli ribonuclease HI by cavity-filling mutations within a hydrophobic core.
AU Ishikawa, Kohki; Nakamura, Haruki; Morikawa, Kosuke; Kanaya, Shigenori (1)
CS (1) Protein Eng. Res. Inst., 6-2-3 Furuedai, Suita, Osaka 565 Japan
SO Biochemistry, (1993) Vol. 32, No. 24, pp. 6171-6178.
ISSN: 0006-2960.
DT Article
LA English
AB The crystal structure of Escherichia coli ribonuclease HI has a cavity near Val-74 within the protein core. In order to fill the cavity space, we constructed two mutant proteins, V74L and V74I, in which Val-74 was replaced with either Leu or Ile, respectively. The mutant proteins are stabilized, as revealed by a 2.1-3.7 degree C increase in the T-m values, as compared to the wild-type protein at pH values of 3.0 and 5.5. The mutant protein V74A, in which Val-74 is replaced with Ala, was also constructed to analyze the reverse effect. The stability of V74A decreases by 7.6 degree C at pH 3.0 and 12.7 degree C at pH 5.5 in T-m as compared to those values for the wild-type protein. None of the three mutations significantly affect the enzymatic activity. The crystal structures of V74L and V741, determined at 1.8- ANG resolution, are almost identical to that of the wild-type protein, except for the mutation site. In the two mutant proteins, calculation by the Voronoi procedure shows that the cavity volumes around the individual mutation sites are remarkably reduced as compared to that in the wild-type protein. These results indicate that the introduction of a methylene group into the cavity, without causing steric clash, contributes to an increase in the hydrophobic interaction within the protein core and thereby enhances protein stability. We also discuss the role of the Leu side chain, which can assume many different local conformations on a helix without sacrificing thermostability.

L30 ANSWER 22 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1994:76706 BIOSIS
DN PREV199497089706
TI Identification of human T cell epitopes in the Mycobacterium leprae heat shock protein 70-kD antigen.
AU Adams, E. (1); Britton, W. J.; Morgan, A.; Goodsall, A. L.; Basten, A.
CS (1) Centenary Inst. Cancer Med. Cell Biol., Locked Bag No. 6, Newtown NSW 2041 Australia

SO Clinical and Experimental Immunology, (1993) Vol. 94, No. 3, pp. 500-506. ISSN: 0009-9104.
DT Article
LA English
AB In a number of pathogens, heat shock proteins (hsp) stimulate humoral and cellular immune responses despite significant sequence identity with host hsp. The 70-kD hsp of Mycobacterium leprae, which shares 47% identity with human hsp70 at the protein level, elicited a T cell response in most Myco. bovis (bacille Calmette-Guerin (BCG)) vaccinees as well as leprosy and **tuberculosis** patients and their contacts. In order to locate T cell epitopes, DNA fragments encoding portions of the 70-kD hsp were expressed in the vector pGEX-2T and tested for T cell reactivity in an in vitro proliferative assay. Cultures of peripheral blood mononuclear cells (PBMC) from BCG vaccinees indicated that the C-terminal half of the molecule contained multiple T cell epitopes, as the T cells from a majority of Myco. leprae hsp70-reactive individuals responded to C-344. Lower proportions of patients with paucibacillary leprosy (36%) and **tuberculosis** patients (16%) responded to C-344. The smaller C-142 fragment which includes the terminal 70 residues unique to Myco. leprae and is the target for the human antibody response elicited a cellular response in few patients and no vaccinees. In order to map T cell epitopes, two series of synthetic peptides encompassing the region 278-502 were prepared. Using overlapping 12mer and 20mer peptides, this region of the molecule was found to contain several potential T cell epitopes. The longer peptides gave a clearer indication of reactive sequences including regions of the molecule which were not identified with the 12mer peptides. Fine **mapping** of reactive **peptide** pools using the 12mer peptides identified two T cell epitopes. Although both were located in regions of the molecule shared with Myco. **tuberculosis**, one appeared to be crossreactive with the equivalent human sequence, and thus has the potential to initiate autoimmune responses.

L30 ANSWER 23 OF 30 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 92099096 EMBASE
DN 1992099096
TI Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of Mycobacterium **tuberculosis**: Evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity responses.
AU Ashbridge K.R.; Backstrom B.T.; Liu H.-X.; Vikerfors T.; Englebretsen D.R.; Harding D.R.K.; Watson J.D.
CS Molecular Medicine Department, University of Auckland, School of Medicine,Auckland, New Zealand
SO Journal of Immunology, (1992) 148/7 (2248-2255). ISSN: 0022-1767 CODEN: JOIMA3
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LA English
SL English
AB In vivo and in vitro T cell responses to overlapping 20-mer peptides that span the entire 19-kDa protein of Mycobacterium **tuberculosis** have been compared in three different strains of mice. Immunization of the mice with peptides and analysis of specific antibody production is an in vivo assay of Th cell activity. Peptides 1-20 and 61-80 elicited strong IgG1 responses in BALB/cJ, C57BL/10J, and B10.BR mice, indicating that these peptides could stimulate Th cells, possibly of a Th2 phenotype. T cells isolated from peptide-immunized mice were challenged in vitro with peptide, and their proliferative responses were analyzed. T cells from these three strains of mice immunized with peptides 1-20, 61-80, and 76-95 also responded to challenge with specific peptide in vitro. In addition,

B10.BR mice and BALB/cJ mice showed antibody and T cell proliferative responses to peptides 136-155 and 145-159, respectively. Thus, in vitro proliferating T cells were found to possess specificities for peptide epitopes that were almost identical to those of the antibody-producing cells. Delayed-type hypersensitivity (DTH) responses to these peptides were also examined in the three strains. Interestingly, the T cells responding in the DTH assay had Ag specificities that were quite different from those identified in the antibody and proliferation assays. These results suggested that DTH Th cells form a separate population from antibody Th and proliferative T cells and these populations of cells were differentially activated, in an Ag-specific manner.

L30 ANSWER 24 OF 30 MEDLINE
AN 92386764 MEDLINE
DN 92386764 PubMed ID: 1381300
TI Characterization of B cell epitopes on the 16K antigen of Mycobacterium **tuberculosis**.
AU Verbon A; Hartskeerl R A; Moreno C; Kolk A H
CS N. H. Swellengrebel Laboratory of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands.
SO CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1992 Sep) 89 (3) 395-401. Journal code: 0057202. ISSN: 0009-9104.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199210
ED Entered STN: 19921023
Last Updated on STN: 19960129
Entered Medline: 19921007
AB To characterize the antigenic parts of the 16K protein of Mycobacterium **tuberculosis**, overlapping peptides according to the amino acid sequence of the 16K protein were synthesized. In total, 14 peptides of 20 amino acids in length with an overlap of 10 amino acids and two additional decapeptides (amino acids 31-40 and 61-70) were tested with eight anti-16K MoAbs and human sera. The common recognition site of MoAbs F67-8 and F67-16 was LRPTFDTRLM (amino acids 31-40) and of MoAbs F159-1 and F159-11 DPDKDVDIMV (amino acids 61-70). However, for binding of the MoAbs to these peptides additional amino acids were required at either the N- or C-terminus, suggesting that some kind of conformation is required. The recognition sites of the MoAbs F23-41, F23-49, F24-2 and TB68 could not be identified using the peptides, indicating that the MoAbs only bound to conformational epitopes and not to peptides which may contain parts of these epitopes. The MoAbs bound to beta-galactosidase fusion proteins comprising parts of the 16K protein, indicating that some kind of native conformation is present on the recombinant proteins. Sera from 14 of 19 patients with **tuberculosis** and none from 19 controls reacted with the purified 16K protein. Sera from four of these 14 patients reacted with two overlapping peptides (amino acids 71-100). Apparently, antibodies in patients' sera against the 16K protein are predominantly directed against conformational epitopes.

L30 ANSWER 25 OF 30 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1990-06219 BIOTECHDS
TI Epitope mapping of the Mycobacterium bovis secretory protein MPB70 using overlapping peptide analysis;
potential application in cattle **tuberculosis** diagnosis; DNA sequence
AU Radford A J; Wood P D; Billman-Jacobe H; Geysen H M; Mason T J; Tribbick G
LO Commonwealth Scientific and Industrial Research Organization, Division of Animal Health, Private Bag no.1, PO, Parkville, Victoria 3052, Australia.
SO J.Gen.Microbiol.; (1990) 136, Pt.2, 265-72

CODEN: JGMIAN
DT Journal
LA English
AB Mycobacterium bovis An5 DNA was partially digested with Sau3A and size-selected on agarose for fragments greater than 10 kb. These fragments were used to construct a gene bank in phage lambda EMBL3 which was then used to establish a restriction map of the secretory protein MPB70 region of the M. bovis chromosome. After subcloning, the complete DNA sequence and predicted protein sequence were determined and, from this information, a series of overlapping octapetides encoding all possible linear epitopes of 8 or less amino acids was synthesized. These peptides were probed with monoclonal antibodies specific for M. bovis and with sera from M. bovis-infected cattle. Epitopes were analyzed for the significance of each amino acid to the antibody binding reaction by replacement net assay. The network analysis suggested an epitope of QDPV for the SB10 monoclonal antibody and an epitope of XNNPE for the polyclonal sera. Epitope mapping may identify specific-specific epitopes within cross-reactive antigens suitable for inclusion in a serological test for cattle **tuberculosis**. (37 ref)

L30 ANSWER 26 OF 30 MEDLINE
AN 89307568 MEDLINE
DN 89307568 PubMed ID: 2545626
TI Structure and mapping of antigenic domains of protein antigen b, a 38,000-molecular-weight protein of Mycobacterium **tuberculosis**.
AU Andersen A B; Hansen E B
CS Mycobacteria Department, Statens Seruminstitut, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (1989 Aug) 57 (8) 2481-8.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198908
ED Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19890818
AB Only a limited number of proteins from Mycobacterium **tuberculosis** have so far been shown to possess species-specific epitopes as defined by monoclonal antibodies. One such protein is protein antigen b (Pab) of molecular weight 38,000, which binds the monoclonal antibodies HYT 28, HAT 2, HBT 12, HGT 3, TB 71, and TB 72. The gene encoding this protein was isolated from a lambda gt11 M. **tuberculosis** DNA library. The nucleotide sequence of the recombinant mycobacterial insert was determined, and an open reading frame of 374 amino acids was identified. The amino acid sequence exhibited 30% homology to a phosphate-binding protein, PstS, from Escherichia coli. The pab gene was subcloned into pBR322 in conjunction with the lacZ gene, and deletions were obtained from the 3' end. The anti-Pab monoclonal antibodies were used to probe crude protein lysates of E. coli transformed with the deletion plasmids. The monoclonal antibodies showed two reactivity patterns; one group of antibodies were dependent on the presence of the ultimate 91 amino acids of the protein, whereas another group of antibodies recognized an antigenic domain located on the middle portion of the molecule. None of the antibodies bound to the N-terminal 117-amino-acid peptide.

L30 ANSWER 27 OF 30 MEDLINE
AN 89381342 MEDLINE
DN 89381342 PubMed ID: 2476491
TI The mapping of epitopes of the 18-kDa protein of Mycobacterium leprae recognized by murine T cells in a proliferation assay.
AU Harris D P; Backstrom B T; Booth R J; Love S G; Harding D R; Watson J D
CS Department of Immunobiology, School of Medicine, University of Auckland,

New Zealand.
SO JOURNAL OF IMMUNOLOGY, (1989 Sep 15) 143 (6) 2006-12.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 198910
ED Entered STN: 19900309
Last Updated on STN: 19960129
Entered Medline: 19891018
AB The 18-kDa protein of Mycobacterium leprae was purified from recombinant plasmids pUL108 and pML-3 grown in Saccharomyces cerevisiae and Escherichia coli, respectively. Significant lymphoproliferative responses were observed when T cells from immunized mice were challenged in culture with purified 18-kDa protein. Synthetic peptides have been prepared that span most of the 148 amino acid residues that constitute the sequence of the 18-kDa protein and used to map epitopes recognized by T cells. When mice were immunized with 18-kDa protein and lymph node cells subsequently prepared and challenged in microculture proliferative assays by using synthetic peptides, only one region of the intact protein appeared stimulatory. This T cell epitope was located between residues 116 and 121, adjacent to an epitope between residues 110 and 115 which we have previously shown to bind the L5 mAb. Immunization of mice with peptides, and subsequent challenge of lymph node cells in assays by using the 18-kDa protein as Ag revealed that residues 111-125 were the most effective in priming responses. Furthermore, the ability of 18-kDa primed lymph node cells to recognize determinants on both M. leprae and Mycobacterium **tuberculosis** indicates that in addition to possessing an M. leprae-specific B cell determinant, the 18-kDa protein contains a cross-reactive T cell epitope(s).

L30 ANSWER 28 OF 30 MEDLINE
AN 89009807 MEDLINE
DN 89009807 PubMed ID: 2459228
TI Epitopes of the Mycobacterium **tuberculosis** 65-kilodalton protein antigen as recognized by human T cells.
AU Oftung F; Mustafa A S; Shinnick T M; Houghten R A; Kvalheim G; Degre M; Lundin K E; Godal T
CS Laboratory for Immunology, Norwegian Radium Hospital, Oslo.
SO JOURNAL OF IMMUNOLOGY, (1988 Oct 15) 141 (8) 2749-54.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 198811
ED Entered STN: 19900308
Last Updated on STN: 19960129
Entered Medline: 19881109
AB A synthetic peptide approach has been used to identify the epitopes recognized by clonal and polyclonal human T cells reactive to the recombinant mycobacterial 65-kDa protein Ag. Three of the four epitopes identified were recognized as cross-reactive between Mycobacterium **tuberculosis** and Mycobacterium leprae, although their amino acid sequence in two of three cases was not identical. The peptide (231-245) defining an epitope recognized as specific to the M. **tuberculosis** complex contains two substitutions compared with the homologous M. leprae region of which one or both are critical to T cell recognition. The reactive T cell clones showed helper/inducer phenotype (CD4+, CD8-), and secrete IL-2, granulocyte-macrophage-CSF, and IFN-gamma upon Ag stimulation. The same clones display cytotoxicity against macrophages pulsed with the relevant peptides or mycobacteria.

L30 ANSWER 29 OF 30 MEDLINE
AN 88226944 MEDLINE
DN 88226944 PubMed ID: 2453469
TI Use of recombinant antigens expressed in Escherichia coli K-12 to map B-cell and T-cell epitopes on the immunodominant 65-kilodalton protein of Mycobacterium bovis BCG.
AU Thole J E; van Schooten W C; Keulen W J; Hermans P W; Janson A A; de Vries R R; Kolk A H; van Embden J D
CS Laboratory for Bacteriology, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.
SO INFECTION AND IMMUNITY, (1988 Jun) 56 (6) 1633-40.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198806
ED Entered STN: 19900308
Last Updated on STN: 19960129
Entered Medline: 19880630
AB In gene libraries of Mycobacterium bovis BCG, Mycobacterium **tuberculosis**, and Mycobacterium leprae, recombinants were frequently encountered that expressed an immunodominant 65-kilodalton (kDa) protein antigen that was shown to react with a high proportion of mycobacterium-reactive human and murine T cells and murine monoclonal antibodies. In this study, recombinant antigens were used to map T-cell and B-cell epitopes on the M. bovis BCG 65-kDa protein that was previously designated MbaA. Four different T-cell-epitope-containing regions (amino acid residues 1 through 16, 17 through 61, 85 through 108, and 235 through 279) were defined that were recognized by seven T-cell clones from patients with tuberculoid leprosy. These regions are distinct from two previously described T-cell epitopes recognized by T cells from a **tuberculosis** patient. As T-cell clones restricted by different class II determinants were shown to be specific for different regions on the 65-kDa protein, the presented data suggested that the products of different human leukocyte antigen class II loci and alleles present different parts of MbaA to the immune system. B-cell epitopes recognized by 20 monoclonal antibodies were assigned to eight different regions of MbaA. Using 15 of these antibodies, we previously showed that MbaA was antigenically related to a common antigen present in many bacterial species. The dispersed localization of the involved epitopes defined here shows that various different parts of MbaA are indeed conserved. These results show that well-defined recombinant antigens are useful tools for the localization of both B- and T-cell-epitope-containing regions of a protein. Peptides synthesized from the sequences of such regions may then exactly define the epitopes relevant for the development of specific diagnostic tests or of vaccines against mycobacteria.

L30 ANSWER 30 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 1973:522783 CAPLUS
DN 79:122783
TI New type of microheterogeneity of blood plasma albumin
AU Troitskii, G. V.; Bagdasar'yan, S. N.
CS Crimean Med. Inst., Simferopol, USSR
SO Byulleten Eksperimental'noi Biologii i Meditsiny (1973), 76(8), 48-50
CODEN: BEBMAE; ISSN: 0365-9615
DT Journal
LA Russian
AB A new type of microheterogeneity of electrophoretically purified plasma albumin was reflected in its soly. in 80% EtOH following pptn. with 10% Cl3CCO2H. The microheterogeneity, confirmed by spectropolarimetry and **peptide mapping**, is probably assocd. with the ability of

LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961217

AB The recall of long-lived immunity in a mouse model of **tuberculosis** (TB) is defined as an accelerated accumulation of reactive T cells in the target organs. We have recently identified Ag 85B and a 6-kilodalton early secretory antigenic target, designated **ESAT-6**, as key antigenic targets recognized by these cells. In the present study, preferential recognition of the **ESAT-6** Ag during the recall of immunity was found to be shared by five of six genetically different strains of mice. Overlapping peptides spanning the sequence of **ESAT-6** were used to map two T cell epitopes on this molecule. One epitope recognized in the context of H-2b,d was located in the N-terminal part of the molecule, whereas an epitope recognized in the context of H-2a,k covered amino acids 51 to 60. Shorter versions of the N-terminal epitope allowed the precise definition of a 13-amino acid core sequence recognized in the context of H-2b. The peptide covering the N-terminal epitope was immunogenic, and a T cell response with the same fine specificity as that induced during TB infection was generated by immunization with the peptide in IFA. In the C57BL/6j strain, this single epitope was recognized by an exceedingly high frequency of splenic T cells (approximately 1:1000), representing 25 to 35% of the total culture filtrate-reactive T cells recruited to the site of infection during the first phase of the recall response. These findings emphasize the relevance of this Ag in the immune response to TB and suggest that immunologic recognition in the first phase of infection is a highly restricted event dominated by a limited number of T cell clones.

results had a weaker relation with exposure (1.9 (1.0-3.5), p=0.05). By contrast, ELISPOT results were not correlated with BCG vaccination status (p=0.7), whereas TST results were significantly more likely to be positive in BCG-vaccinated contacts (12.1 (1.3-115.7), p=0.03). Interpretation: This new antigen-specific T cell-based assay could allow more accurate identification of symptom-free individuals recently exposed to M tuberculosis, and thereby help to improve tuberculosis control.

L6 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2001:235090 BIOSIS
DN PREV200100235090
TI Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells.
AU **Lalvani, Ajit (1)**; **Pathan, Ansar A.**; McShane, Helen; Wilkinson, Robert J.; Latif, Mohammed; Conlon, Christopher P.; Pasvol, Geoffrey; Hill, Adrian V. S.
CS (1) Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Level 7, Oxford, OX3 9DU: ajit.lalvani@ndm.ox.ac.uk UK
SO American Journal of Respiratory and Critical Care Medicine, (March, 2001) Vol. 163, No. 4, pp. 824-828. print.
ISSN: 1073-449X.
DT Article
LA English
SL English
AB There is no reliable means of detecting latent M. tuberculosis infection, and even in patients with active tuberculosis, infection is often unconfirmed. We hypothesized that M. tuberculosis antigen-specific T cells might reliably indicate infection. We enumerated peripheral blood-derived interferon gamma (IFN-gamma)-secreting T cells responding to epitopes from **ESAT-6**, an antigen that is highly specific for M. tuberculosis complex but absent from BCG, in four groups of individuals. Forty-five of 47 patients with bacteriologically confirmed tuberculosis had **ESAT-6**-specific IFN-gamma-secreting T cells, compared with four of 47 patients with nontuberculous illnesses, indicating that these T cells are an accurate marker of M. tuberculosis infection. This assay thus has a sensitivity of 96% (95% confidence interval (CI) 92-100) for detecting M. tuberculosis infection in this patient population. By comparison, of the 26 patients with tuberculosis who had a diagnostic tuberculin skin test (TST), only 18 (69%) were positive (p = 0.003). In addition, 22 of 26 (85%) TST-positive exposed household contacts had **ESAT-6**-specific T cells, whereas zero of 26 unexposed BCG-vaccinated subjects responded. This approach enables rapid detection of M. tuberculosis infection in patients with active tuberculosis and in exposed asymptomatic individuals at high risk of latent infection; it also successfully distinguishes between M. tuberculosis infection and BCG vaccination. This capability may facilitate tuberculosis control in nonendemic regions.

L6 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 2001:116597 BIOSIS
DN PREV200100116597
TI Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians.
AU **Lalvani, Ajit (1)**; Nagvenkar, Punam; Udwadia, Zarir; **Pathan, Ansar A.**; Wilkinson, Katalin A.; Shastri, Jayanthi S.; Ewer, Katie; Hill, Adrian V. S.; Mehta, Ajita; Rodrigues, Camilla
CS (1) Nuffield Dept. of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Level 7, Oxford, OX3 9DU: ajit.lalvani@ndm.ox.ac.uk UK

SO Journal of Infectious Diseases, (1 February, 2001) Vol. 183, No. 3, pp. 469-477. print.
ISSN: 0022-1899.
DT Article
LA English
SL English
AB Knowledge of the prevalence of latent Mycobacterium tuberculosis infection is crucial for effective tuberculosis control, but tuberculin skin test surveys have major limitations, including poor specificity because of the broad antigenic cross-reactivity of tuberculin. The M. tuberculosis RD1 genomic segment encodes proteins, such as early secretory antigenic target (**ESAT**)-**6**, that are absent from M. bovis bacille Calmette-Guerin (BCG) and most environmental mycobacteria. We recently identified circulating **ESAT-6**-specific T cells as an accurate marker of M. tuberculosis infection. Here, interferon-gamma-secreting T cells specific for peptides derived from **ESAT-6** and a second RD1 gene product, CFP10, were enumerated in 100 prospectively recruited healthy adults in Bombay (Mumbai), India. Eighty percent responded to gtoreq1 antigen, and many donors had high frequencies of T cells that were specific for certain immunodominant peptides. In contrast, of 40 mostly BCG-vaccinated, United Kingdom-resident healthy adults, none responded to either antigen. This study suggests an 80% prevalence of latent M. tuberculosis infection in urban India.

L6 ANSWER 8 OF 12 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 7
AN 2000-365579 [31] WPIDS
DNN N2000-273545 DNC C2000-110441
TI Novel method of diagnosing infection, or exposure of a host, to a mycobacterium comprising contacting T cells from the host with **ESAT-6** derived peptides.
DC B04 D16 S03
IN **LALVANI, A**; PATHAN, A A; AJIT, L; ANSAR, A P
PA (ISIS-N) ISIS INNOVATION LTD
CYC 91
PI WO 2000026248 A2 20000511 (200031)* EN 33p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9964809 A 20000522 (200040)
BR 9915055 A 20010807 (200152)
EP 1144447 A2 20011017 (200169) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ZA 2001003356 A 20020327 (200230) 51p
CN 1350546 A 20020522 (200258)
JP 2002532064 W 20021002 (200279) 48p
ADT WO 2000026248 A2 WO 1999-GB3635 19991103; AU 9964809 A AU 1999-64809 19991103; BR 9915055 A BR 1999-15055 19991103, WO 1999-GB3635 19991103; EP 1144447 A2 EP 1999-952697 19991103, WO 1999-GB3635 19991103; ZA 2001003356 A ZA 2001-3356 20010425; CN 1350546 A CN 1999-813005 19991103; JP 2002532064 W WO 1999-GB3635 19991103, JP 2000-579635 19991103
FDT AU 9964809 A Based on WO 200026248; BR 9915055 A Based on WO 200026248; EP 1144447 A2 Based on WO 200026248; JP 2002532064 W Based on WO 200026248
PRAI US 1998-107004P 19981104; GB 1998-24213 19981104
AB WO 200026248 A UPAB: 20000630
NOVELTY - Diagnosing infection in a host (M), or exposure of a host, to a mycobacterium which expresses **ESAT-6**, comprises contacting T cells from the host with at least 1 of 11 peptides ((I)-(XI)) of 15 amino acids (aa), or their analogues which bind a T cell receptor that binds (I)-(XI), but not peptides (III) or (V) (or their analogues)

searches revealed that the three members of the TB10.4 family were present only in strains of the Mycobacterium **tuberculosis** complex, including BCG, and M. kansasii, whereas other atypical mycobacteria had either one (M. avium, M. intracellulare, and M. marinum) or none (M. scrofulaceum, M. fortuitum, and M. szulgai) of the genes. The fine specificity of the T-cell response to the three closely related **esat-6** family members was markedly different, with only a few epitopes shared between the molecules. Minimal differences in the amino acid sequence translated into large differences in recognition by T cells and secretion of gamma interferon. In general, the peptides from TB10.4 stimulated the largest responses, but epitopes unique to both TB10.3 and TB12.9 were found. The relevance of the findings for TB vaccine development and as a potential mechanism for immune evasion is discussed.

L10 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS
AN 2000:282543 CAPLUS
DN 133:41846
TI Antigen specificity in experimental bovine **tuberculosis**
AU Rhodes, S. G.; Gavier-Widen, D.; Buddle, B. M.; Whelan, A. O.; Singh, M.; Hewinson, R. G.; Vordermeier, H. M.
CS TB Research Group, Veterinary Laboratories Agency, Surrey, KT15 3NB, UK
SO Infection and Immunity (2000), 68(5), 2573-2578
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB This report describes the kinetics of T-cell responses to a panel of mycobacterial antigens (PPD-M, PPD-A, **ESAT-6**, Ag85, 38kD, MPB64, MPB70, MPB83, hsp16.1, hsp65, and hsp70) following exptl. infection of cattle with Mycobacterium bovis. Increased antigen-specific lymphocyte proliferation, gamma interferon, and interleukin-2 responses were obsd. in all calves following infection. Pos. lymphocyte proliferation and cytokine responses to PPD-M and **ESAT-6** were obsd. throughout the infection period studied. In contrast, responses to all other antigens were more variable and were not constantly present, suggesting that antigen cocktails rather than individual antigens should be used for immunodiagnosis. The detection of cytokine responses in the absence of lymphocyte proliferation, particularly during the early stages of infection, suggests a role for antigen-specific cytokine readout systems in the early identification of M. bovis infection in cattle.
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2000:34137 BIOSIS
DN PREV200000034137
TI **T-cell recognition** of Mycobacterium **tuberculosis** culture filtrate fractions in **tuberculosis** patients and their household contacts.
AU Demissie, Abebech; Ravn, Pernille; Olobo, Joseph; Doherty, T. Mark; Eguale, Tewodros; Geletu, Mulu; Hailu, Wondewossen; Andersen, Peter (1); Britton, Sven
CS (1) Statens Seruminstitut, 5 Artillerivej, Copenhagen, 2300 S Denmark
SO Infection and Immunity, (Nov., 1999) Vol. 67, No. 11, pp. 5967-5971.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB We examined the immune responses of patients with active pulmonary **tuberculosis** (TB) and their healthy household contacts to short-term culture filtrate (ST-CF) of Mycobacterium **tuberculosis** or molecular mass fractions derived from it. Our goal was to identify fractions strongly recognized by donors and differences among the donor

Vordermeier et al

Addlestone, Surrey, KT15 3NB, UK: mvordermeier.vla@gtnet.gov.uk UK
SO Infection and Immunity, (April 2003, 2003) Vol. 71, No. 4, pp. 1980-1987. print.
ISSN: 0019-9567.
DT Article
LA English
AB Bioinformatics tools have the potential to accelerate research into the design of vaccines and **diagnostic** tests by exploiting genome sequences. The aim of this study was to assess whether in silico analysis could be combined with in vitro screening methods to rapidly identify peptides that are immunogenic during Mycobacterium bovis infection of cattle. In the first instance the M. bovis-derived protein **ESAT-6** was used as a model antigen to describe peptides containing **T-cell** epitopes that were frequently **recognized** across mammalian species, including natural hosts for **tuberculosis** (humans and cattle) and small-animal models of **tuberculosis** (mice and guinea pigs). Having demonstrated that some peptides could be recognized by T cells from a number of M. bovis-infected hosts, we tested whether a virtual-matrix-based human prediction program (ProPred) could identify peptides that were recognized by T cells from M. bovis-infected cattle. In this study, 73% of the experimentally defined peptides from 10 M. bovis antigens that were recognized by bovine T cells contained motifs predicted by ProPred. Finally, in validating this observation, we showed that three of five peptides from the mycobacterial antigen Rv3019c that were predicted to contain HLA-DR-restricted epitopes were recognized by T cells from M. bovis-infected cattle. The results obtained in this study support the approach of using bioinformatics to increase the efficiency of epitope screening and selection.

L13 ·ANSWER 2 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2003:152704 BIOSIS
DN PREV200300152704
TI Human Th1 cell lines recognize the Mycobacterium **tuberculosis** **ESAT-6** antigen and its peptides in association with frequently expressed HLA class II molecules.
AU Mustafa, A. S. (1); Shaban, F. A.; Al-Attiyah, R.; Abal, A. T.; El-Shamy, A. M.; Andersen, P.; Oftung, F.
CS (1) Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat, 13110, Kuwait: abusalim@hsc.kuniv.edu.kw Kuwait
SO Scandinavian Journal of Immunology, (February 2003, 2003) Vol. 57, No. 2, pp. 125-134. print.
ISSN: 0300-9475.
DT Article
LA English
AB We have used a synthetic-peptide approach to map epitope regions of the Mycobacterium **tuberculosis** **ESAT-6** antigen recognized by human T cells in relation to major histocompatibility complex (MHC) restriction. **ESAT-6**-specific CD4+ T-cell lines were established by stimulating peripheral blood mononuclear cells from 25 HLA-DR-typed **tuberculosis** patients with complete antigen in vitro. The established T-cell lines were then screened for proliferation and interferon-gamma (IFN-gamma) secretion in response to eight overlapping 20-mer peptides covering the **ESAT-6** sequence. The response of the T-cell lines to **ESAT-6** and peptides from a human leucocyte antigen (HLA)-heterogeneous group of donors suggested the presence of multiple epitopes and promiscuous recognition of the antigen. Analysis of antigen and peptide recognition in the presence of anti-HLA class I and class II antibodies suggested that the **T-cell** lines **recognized** **ESAT-6** in association with HLA-DR and -DQ molecules. Furthermore, testing of selected T-cell lines with **ESAT-6** and the peptides in the presence of autologous and allogeneic HLA-DR- and -DQ-typed antigen-presenting cells identified HLA-DR2, -DR52 and -DQ2

Rhodes et al

Hewinson, R. G.; Vordermeier, H. M.
CS TB Research Group, Veterinary Laboratories Agency, Surrey, KT15 3NB, UK
SO Infection and Immunity (2000), 68(5), 2573-2578
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB This report describes the kinetics of T-cell responses to a panel of mycobacterial antigens (PPD-M, PPD-A, **ESAT-6**, Ag85, 38kD, MPB64, MPB70, MPB83, hsp16.1, hsp65, and hsp70) following exptl. infection of cattle with Mycobacterium bovis. Increased antigen-specific lymphocyte proliferation, gamma interferon, and interleukin-2 responses were obsd. in all calves following infection. Pos. lymphocyte proliferation and cytokine responses to PPD-M and **ESAT-6** were obsd. throughout the infection period studied. In contrast, responses to all other antigens were more variable and were not constantly present, suggesting that antigen cocktails rather than individual antigens should be used for immunodiagnosis. The **detection** of cytokine responses in the absence of lymphocyte proliferation, particularly during the early stages of infection, suggests a role for antigen-specific cytokine readout systems in the early identification of M. bovis infection in cattle.
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1998:120633 BIOSIS
DN PREV199800120633
TI B-cell epitopes and quantification of the **ESAT-6** protein of Mycobacterium **tuberculosis**.
AU Harboe, Morten (1); Malin, Adam S.; Dockrell, Hazel S.; Wiker, Harald Gotten; Ulvund, Gunni; Holm, Arne; Jorgensen, Mikala Clok; Andersen, Peter
CS (1) Inst. Immunol. Rheumatol., Univ. Oslo, Fr. Qvams gate 1, N-0172 Oslo Norway
SO Infection and Immunity, (Feb., 1998) Vol. 66, No. 2, pp. 717-723.
ISSN: 0019-9567.
DT Article
LA English
AB **ESAT-6** is an important **T-cell** antigen **recognized** by protective T cells in animal models of infection with Mycobacterium **tuberculosis**. In an enzyme-linked immunosorbent **assay** (ELISA) with overlapping peptides spanning the sequence of **ESAT-6**, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the molecule. Assays with synthetic truncated peptides allowed a precise mapping of the epitope to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two additional areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native **ESAT-6**. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The **assay** was suitable for quantification of **ESAT-6** in M. **tuberculosis** antigen preparations, showing no reactivity with M. bovis BCG Tokyo culture fluid, used as a negative control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of **ESAT-6** expression from vaccinia virus constructs containing the **esat-6** gene; this expression could not be identified by standard immunoblotting.

SO JOURNAL OF IMMUNOLOGY, (2001 Nov 1) 167 (9) 5217-25.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200112
ED Entered STN: 20011024
Last Updated on STN: 20020122
Entered Medline: 20011205
AB The wide spectrum of clinical outcomes following infection with Mycobacterium **tuberculosis** is largely determined by the host immune response; therefore, we studied several clinically defined groups of individuals (n = 120) that differ in their ability to contain the bacillus. To quantitate M. **tuberculosis**-specific T cells directly ex vivo, we enumerated IFN-gamma-secreting CD4 T cells specific for **ESAT-6**, a secreted Ag that is highly specific for M. **tuberculosis**, and a target of protective immune responses in animal models. We found that frequencies of circulating **ESAT-6** peptide-specific IFN-gamma-secreting CD4 T cells were higher in latently infected healthy contacts and subjects with minimal disease and low bacterial burdens than in patients with culture-positive active pulmonary **tuberculosis** ($p = 0.009$ and $p = 0.002$, respectively). Importantly, the frequency of these Ag-specific CD4 T cells fell progressively in all groups with **treatment** ($p = 0.005$), suggesting that the lower responses in patients with more extensive disease were not due to **tuberculosis**-induced immune suppression. This population of M. **tuberculosis** Ag-specific Th1-type CD4 T cells appears to correlate with clinical phenotype and declines during successful therapy; these features are consistent with a role for these T cells in the containment of M. **tuberculosis** in vivo. Such findings may assist in the design and evaluation of novel **tuberculosis vaccine** candidates.

L16 ANSWER 19 OF 47 MEDLINE DUPLICATE 7
AN 2001366967 MEDLINE
DN 21321119 PubMed ID: 11427279
TI Protective efficacy against **tuberculosis** of **ESAT-6** secreted by a live Salmonella typhimurium **vaccine** carrier strain and expressed by naked DNA.
AU Mollenkopf H J; Groine-Triebkorn D; Andersen P; Hess J; Kaufmann S H
CS Max-Planck-Institute for Infection Biology, Department of Immunology, Schumannstr. 21/22, 10117 Berlin, Germany.. mollenkopf@mpiib-berlin.mpg.de
SO VACCINE, (2001 Jul 16) 19 (28-29) 4028-35.
Journal code: 8406899. ISSN: 0264-410X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20011015
Last Updated on STN: 20011015
Entered Medline: 20011011
AB We have constructed a recombinant (r) attenuated Salmonella typhimurium strain which secretes **ESAT-6** of Mycobacterium **tuberculosis** via the hemolysin secretion system of E. coli. Additionally, we have ligated **ESAT-6** to different commercially available mammalian expression systems for use as naked DNA **vaccines**. We studied protection against M. **tuberculosis** induced by **vaccination** with each of these constructs alone or in combination in mice. **Vaccination** with a single dose of r S. typhimurium secreting **ESAT-6** reduced numbers of tubercle bacilli in the lungs throughout the course of infection. The

detection of active TB. DESIGN: We describe five patients with uncommon presentations of **tuberculosis**, in whom the diagnosis was delayed by negative or conflicting results of diagnostic procedures aimed at detection of M. **tuberculosis** and an uninformative tuberculin skin test. IFN-gamma production in response to **ESAT-6** and CFP-10 by peripheral blood mononuclear cells from these patients was evaluated before and during anti-**tuberculosis treatment** . RESULTS: In all five patients, IFN-gamma responses to **ESAT-6** and/or CFP-10 were above the cut-off level defined in a previous study. During **treatment**, IFN-gamma responses generally increased. CONCLUSION: These results indicate that T cell responses to M. **tuberculosis**-specific antigens have potential diagnostic value when TB is suspected and the results of other diagnostic tests are inconclusive, especially in BCG-**vaccinated** individuals.

L16 ANSWER 25 OF 47 MEDLINE
AN 2001566195 MEDLINE
DN 21525390 PubMed ID: 11669220
TI Antigen discovery and **tuberculosis vaccine** development in the post-genomic era.
AU Louise R; Skjot V; Agger E M; Andersen P
CS Department of TB Immunology, Statens Serum Institut, Copenhagen, Denmark.
SO SCANDINAVIAN JOURNAL OF INFECTIOUS DISEASES, (2001) 33 (9) 643-7. Ref: 43
Journal code: 0215333. ISSN: 0036-5548.
CY Sweden
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20011024
Last Updated on STN: 20020302
Entered Medline: 20020301
AB For a number of years, a major effort has been put into the identification of candidate molecules for inclusion in a novel **vaccine** against **tuberculosis**. Various techniques have been exploited and have resulted in the identification of immunologically important antigens such as the immunodominant antigens **ESAT-6** and antigen 85A/B. Today, the availability of the total nucleotide sequence of the Mycobacterium **tuberculosis** genome enables a post-genomic antigen discovery approach based on denotation and screening of complete protein families containing immunodominant molecules. One group of genes sharing properties with **ESAT-6** constitute what has been called the **esat-6** gene family. The genes have 10-35% homology to **esat-6**, are approximately the same size and share genomic organization. The data accumulated so far demonstrate that these molecules are immunodominant antigens strongly recognized in human TB patients and with the potential for a novel TB **vaccine**.

L16 ANSWER 26 OF 47 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 2001:326659 BIOSIS
DN PREV200100326659
TI Use of synthetic peptides derived from the antigens **ESAT-6** and CFP-10 for differential diagnosis of bovine **tuberculosis** in cattle.
AU Vordermeier, H. M. (1); Whelan, A.; Cockle, P. J.; Farrant, L.; Palmer, N.; Hewinson, R. G.
CS (1) TB Research Group, Department of Bacterial Diseases, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, KT15 3NB: mvordermeier.vla@gtnet.gov.uk UK
SO Clinical and Diagnostic Laboratory Immunology, (May, 2001) Vol. 8, No. 3,

pp. 571-578. print.
ISSN: 1071-412X.
DT Article
LA English
SL English
AB In Great Britain an independent scientific review for the government has concluded that the development of a cattle **vaccine** against Mycobacterium bovis infection holds the best long-term prospect for **tuberculosis** control in British herds. A precondition for **vaccination** is the development of a complementary diagnostic test to differentiate between **vaccinated** animals and those infected with M. bovis so that testing and slaughter-based control strategies can continue alongside **vaccination**. To date bacillus Calmette-Guerin (BCG), an attenuated strain of M. bovis, is the only available **vaccine** for the **prevention** of **tuberculosis**. However, tests based on tuberculin purified protein derivative cannot distinguish between M. bovis infection and BCG **vaccination**. Therefore, specific antigens expressed by M. bovis but absent from BCG constitute prime candidates for differential diagnostic reagents. Recently, two such antigens, **ESAT-6** and CFP-10, have been reported to be promising candidates as diagnostic reagents for the detection of M. bovis infection in cattle. Here we report the identification of promiscuous peptides of CFP-10 that were recognized by M. bovis-infected cattle. Five of these peptides were formulated into a peptide cocktail together with five peptides derived from **ESAT-6**. Using this peptide cocktail in T-cell assays, M. bovis-infected animals were detected, while BCG-**vaccinated** or Mycobacterium avium-sensitized animals did not respond. The sensitivity of the peptide cocktail as an antigen in a whole-blood gamma interferon assay was determined using naturally infected field reactor cattle, and the specificity was determined using blood from BCG-**vaccinated** and noninfected, nonvaccinated animals. The sensitivity of the assay in cattle with confirmed **tuberculosis** was found to be 77.9%, with a specificity of 100% in BCG-**vaccinated** or nonvaccinated animals. This compares favorably with the specificity of tuberculin when tested in noninfected or **vaccinated** animals. In summary, our results demonstrate that this peptide cocktail can discriminate between M. bovis infection and BCG **vaccination** with a high degree of sensitivity and specificity.

L16 ANSWER 27 OF 47 MEDLINE
AN 2001414836 MEDLINE
DN 21357288 PubMed ID: 11463225
TI Towards more accurate diagnosis of bovine **tuberculosis** using defined antigens.
AU Pollock J M; Buddle B M; Andersen P
CS Veterinary Sciences Division, Stormont, Belfast, UK.
SO Tuberculosis (Edinb), (2001) 81 (1-2) 65-9. Ref: 49
Journal code: 100971555. ISSN: 1472-9792.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200109
ED Entered STN: 20010910
Last Updated on STN: 20021030
Entered Medline: 20010906
AB Diagnostic accuracy is of paramount importance in test-and-slaughter programmes for the eradication of bovine **tuberculosis** (TB). Currently applied methods, such as in vivo skin testing and in vitro interferon-gamma (IFN- gamma) testing, utilize purified protein

6 response was found in TB patients.

L16 ANSWER 30 OF 47 MEDLINE DUPLICATE 11
AN 2000182117 MEDLINE
DN 20182117 PubMed ID: 10715531
TI The immunogenicity of single and combination DNA **vaccines** against **tuberculosis**.
AU Morris S; Kelley C; Howard A; Li Z; Collins F
CS Laboratory of Mycobacteria, Center for Biologics Evaluation and Research, United States Food and Drug Administration, Bethesda, MD 20892, USA.. morris@cber.fda.gov
SO VACCINE, (2000 Apr 14) 18 (20) 2155-63.
Journal code: 8406899. ISSN: 0264-410X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200007
ED Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711
AB DNA immunization is a promising new approach for the development of novel **tuberculosis vaccines**. In this study, the immune responses following the administration of single and combination **tuberculosis** DNA **vaccines** were evaluated. Single DNA **vaccines** encoding the MPT-63 and MPT-83 **tuberculosis** antigens evoked partial protection against an aerogenic challenge with M. **tuberculosis** Erdman in the mouse model of pulmonary **tuberculosis**. Immunization with a multivalent combination DNA **vaccine** (containing the **ESAT-6**, MPT-64, MPT-63, and KatG constructs) generated immune responses that indicated an absence of antigenic competition since antigen-specific cell-mediated and humoral responses were detected to each component of the mixture. More importantly, the combination **vaccine** elicited a strong protective response relative to the protection evoked by live BCG **vaccine**.

L16 ANSWER 31 OF 47 MEDLINE
AN 2000283769 MEDLINE
DN 20283769 PubMed ID: 10823800
TI Detection of active **tuberculosis** infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10.
CM Comment in: J Infect Dis. 2001 Dec 1;184(11):1497-8
AU Arend S M; Andersen P; van Meijgaarden K E; Skjot R L; Subronto Y W; van Dissel J T; Ottenhoff T H
CS Dept. of Infectious Diseases, C5P, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.. smarend@lumc.nl
SO JOURNAL OF INFECTIOUS DISEASES, (2000 May) 181 (5) 1850-4.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200007
ED Entered STN: 20000728
Last Updated on STN: 20030105
Entered Medline: 20000720
AB The purified protein derivative (PPD) skin test has no predictive value for **tuberculosis** (TB) in Mycobacterium bovis bacillus Calmette-Guerin (BCG)-**vaccinated** individuals because of cross-reactive responses to nonspecific constituents of PPD. T cell responses to early-secreted antigenic target 6-kDa protein (**ESAT**

-6) and the newly identified culture filtrate protein 10 (CFP-10), 2 proteins specifically expressed by M. **tuberculosis** (MTB) but not by BCG strains, were evaluated. Most TB patients responded to **ESAT-6** (92%) or CFP-10 (89%). A minority of BCG-**vaccinated** individuals responded to both **ESAT-6** and CFP-10, their history being consistent with latent infection with MTB in the presence of protective immunity. No responses were found in PPD-negative controls. The sensitivity and specificity of the assay were 84% and 100%, respectively, at a cutoff of 300 pg of interferon-gamma/mL. These data indicate that **ESAT-6** and CFP-10 are promising antigens for highly specific immunodiagnosis of TB, even in BCG-**vaccinated** individuals.

L16 ANSWER 32 OF 47 MEDLINE DUPLICATE 12
AN 2000386478 MEDLINE
DN 20354875 PubMed ID: 10898510
TI Efficient protection against Mycobacterium **tuberculosis** by **vaccination** with a single subdominant epitope from the **ESAT-6** antigen.
AU Olsen A W; Hansen P R; Holm A; Andersen P
CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Jun) 30 (6) 1724-32.
Journal code: 1273201. ISSN: 0014-2980.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200008
ED Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000809
AB We have investigated the **vaccine** potential of two peptides derived from the 6-kDa early secretory antigenic target (**ESAT**)-**6** antigen in the mouse model of **tuberculosis**. The peptides were both strongly immunogenic in B6CBAF1 (H-2b,k) mice and primed recall responses of the same intensity after immunization. However, both **tuberculosis** infection and immunization with **ESAT-6** resulted in responses focused towards ESAT-61-20. Multiple antigen peptide constructs as well as free peptides were emulsified with dimethyl dioctadecylammonium bromide/monophosphoryl lipid A/IL-2 and tested as experimental **vaccines** in an i.v. and aerosol model of **tuberculosis** in mice. The peptide were highly immunogenic and induced cellular responses of the same magnitude. However, only **vaccines** based on the subdominant ESAT-651-70 epitope promoted significant levels of protective immunity and the level of protection was equivalent to that achieved with **ESAT-6** and BCG. These findings demonstrate the potential of peptide-based **vaccines** against **tuberculosis** and indicate that there is not direct correlation between the hierarchy of response to naturally processed peptides and their ability to induce protective immunity against Mycobacterium **tuberculosis**.

L16 ANSWER 33 OF 47 MEDLINE
AN 2000417694 MEDLINE
DN 20336500 PubMed ID: 10875803
TI Toward the development of diagnostic assays to discriminate between Mycobacterium bovis infection and bacille Calmette-Guerin **vaccination** in cattle.
AU Vordermeier H M; Cockle P J; Whelan A O; Rhodes S; Hewinson R G
CS Tuberculosis Research Group, Bacteriology Department, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.. mvordermeier.vla@gtnet.gov.uk
SO CLINICAL INFECTIOUS DISEASES, (2000 Jun) 30 Suppl 3 S291-8.

ESAT-6 (6 kDa) as candidate antigens, DNA **vaccines** were prepared and tested for immunogenicity and protective efficacy in a murine model of aerosolized **tuberculosis** (TB). Intramuscular immunization with DNA-64 or DNA-85B resulted in the activation of CD4(+) T cells, which produce gamma interferon (IFN-gamma), and high titers of specific immunoglobulin G antibodies. Further, DNA-64 induced major histocompatibility complex class I-restricted CD8(+) cytotoxic T cells. The addition of a eukaryotic leader sequence to mpt64 did not significantly increase the T-cell or antibody response. Each of the three DNA vectors stimulated a significant reduction in the level of M. **tuberculosis** infection in the lungs of mice challenged 4 weeks after immunization, but not to the levels resulting after immunization with Mycobacterium bovis BCG. The **vaccines** showed a consistent hierarchy of protection, with the most effective being Ag85B, followed by **ESAT-6** and then MPT64. Coimmunization with the three vectors resulted in a greater degree of protection than that induced by any single vector. This protective efficacy was associated with the emergence of IFN-gamma-secreting T cells earlier than in infected animals immunized with a control vector. The efficacy of these DNA **vaccines** suggests that multisubunit **vaccination** may contribute to future **vaccine** strategies against TB.

L16 ANSWER 39 OF 47 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1999:486214 BIOSIS
DN PREV199900486214
TI Development of diagnostic reagents to differentiate between Mycobacterium bovis BCG **vaccination** and M. bovis infection in cattle.
AU Vordermeier, H. M. (1); Cockle, P. C.; Whelan, A.; Rhodes, S.; Palmer, N.; Bakker, D.; Hewinson, R. G.
CS (1) TB Research Group, Bacteriology Department, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, KT15 3NB UK
SO Clinical and Diagnostic Laboratory Immunology, (Sept., 1999) Vol. 6, No. 5, pp. 675-682.
ISSN: 1071-412X.
DT Article
LA English
SL English
AB In Great Britain a recent independent scientific review for the government has concluded that the development of a cattle **vaccine** against Mycobacterium bovis holds the best long-term prospect for **tuberculosis** control in British herds. A sine qua non for **vaccination** is the development of a complementary diagnostic test to differentiate between **vaccinated** animals and those infected with M. bovis so that test-and-slaughter-based control strategies can continue alongside **vaccination**. In order to assess the feasibility of developing a differential diagnostic test for a live **vaccine**, we chose M. bovis BCG Pasteur as a model system. Recombinant forms of antigens which are expressed in M. bovis but not, or only at low levels, in BCG Pasteur (**ESAT-6**, MPB64, MPB70, and MPB83) were produced. These reagents were tested either alone or in combination by using peripheral blood mononuclear cells from M. bovis-infected, BCG-**vaccinated**, and Mycobacterium avium-sensitized calves. All four antigens induced in vitro proliferation and gamma interferon responses only in M. bovis-infected animals. A cocktail composed of **ESAT-6**, MPB64, and MPB83 identified infected animals but not those **vaccinated** with BCG. In addition, promiscuous T-cell epitopes of **ESAT-6**, MPB64, and MPB83 were formulated into a peptide cocktail. In T-cell assays with this peptide cocktail, infected animals were identified with frequencies similar to those obtained in assays with the protein cocktail, while BCG-**vaccinated** or M. avium-sensitized animals did not

immune Africans in The Gambia, eight largely conserved cytotoxic T-lymphocyte epitopes in P. falciparum, restricted by several different HLA class I alleles, were identified. Several epitopes were also recognized in Tanzanians and cytotoxic T-lymphocytes recognized endogenously processed antigen.4.In **tuberculosis** patients with HLA-B52, a CD8+ cytotoxic T-lymphocyte epitope was identified in **ESAT-6**, a secreted antigen specific for M. **tuberculosis** complex but absent in BCG. Cytotoxic T-lymphocytes exhibited HLA-B52-restricted peptide-specific interferon-gamma release and lytic activity and recognized endogenously processed antigen.5.These studies demonstrate that CD8+ cytotoxic T-lymphocytes specific for mycobacterial and protozoal antigens are induced during natural infections in humans. The identification of these T-cells endorses current strategies to develop cytotoxic T-lymphocyte-inducing **vaccines** against P. falciparum and M. **tuberculosis** and highlights candidate antigens for inclusion in subunit **vaccines**.

L16 ANSWER 46 OF 47 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 1998322420 EMBASE
TI Progress towards a new **tuberculosis vaccine**.
AU Lowrie D.B.; Silva C.L.; Tascon R.E.
CS Dr. D.B. Lowrie, Natl. Institute for Medical Research, Ridgeway, Mill Hill, London NW7 1AA, United Kingdom. d-lowrie@nimr.mrc.ac.uk
SO BioDrugs, (1998) 10/3 (201-213).
Refs: 149
ISSN: 1173-8804 CODEN: BIDRF4
CY New Zealand
DT Journal; General Review
FS 004 Microbiology
006 Internal Medicine
015 Chest Diseases, Thoracic Surgery and Tuberculosis
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB New weapons are needed in the fight against **tuberculosis**, both antibacterial drugs and a **vaccine**. If one new antituberculosis drug is developed it will encounter emerging resistance; at least two are needed, to be used in combination only. This is a complicated and difficult goal. In contrast, an effective new **vaccine** would have multiple antigenic targets within the bacterium, making the emergence of resistance to the **vaccine** unlikely. This is a simpler goal to achieve, and recent research indicates that it may be within reach. A diverse range of protein antigens can give encouragingly high levels of protective immunity in animal models when administered with adjuvants or as DNA **vaccines**. Accelerated arrest of bacterial multiplication, followed by sustained decline in bacterial numbers, are key parameters of protection; the **vaccine** must target antigens produced by actively multiplying bacteria as well as growth-inhibited bacteria. Consistent with this, the protective antigens have been found among secreted and stress proteins (for example Ag85, **ESAT-6** , hsp65, hsp70). Species- specific antigens are not required, so these remain available for diagnostic tests. Adoptive transfer of protection from **vaccinated** or infected mice into naive mice by transfer of purified T cells and clones shows that protection is expressed by antigen-specific cytotoxic T cells that produce interferon- .gamma. and lyse infected macrophages. These cells are produced in response to endogenous antigen. DNA **vaccination** appears to be superior to recombinant mycobacterial or viral vectors for this purpose.

L16 ANSWER 47 OF 47 MEDLINE DUPLICATE 16
AN 1998069418 MEDLINE
DN 98069418 PubMed ID: 9406344

TI Genetic **vaccination** against **tuberculosis**.
AU Lowrie D B; Silva C L; Tascon R E
CS National Institute for Medical Research, London, UK.
SO SPRINGER SEMINARS IN IMMUNOPATHOLOGY, (1997) 19 (2) 161-73. Ref: 113
Journal code: 7910384. ISSN: 0172-6641.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; AIDS
EM 199802
ED Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980209
AB New weapons are needed in the fight against **tuberculosis**.
Recent research indicates that a **vaccine** better than BCG may be within reach. A diverse range of protein antigens can give encouragingly high levels of protective immunity in animal models when administered with adjuvants or as DNA **vaccines**. Accelerated arrest of bacterial multiplication followed by sustained decline in bacterial numbers are key parameters of protection and so the **vaccine** must target antigens produced by both actively multiplying and growth-inhibited bacteria. Consistent with this, the protective antigens have been found among secreted and stress proteins (e.g. Ag85, **ESAT-6**, hsp65, hsp70). Species-specific antigens are not needed, hence these remain available for diagnostic tests. Adoptive transfer of protection from **vaccinated** or infected mice into naive mice by transfer of purified T cells and clones shows that protection is expressed by antigen-specific cytotoxic T cells that produce interferon-gamma and lyse infected macrophages. These cells are produced in response to endogenous antigen. DNA **vaccination** appears to be an excellent way of generating these cells and may be able to give long-lasting protection.

=> d his

(FILE 'HOME' ENTERED AT 12:28:34 ON 17 JUL 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 12:28:50 ON 17 JUL 2003

		E LALVANI AJIT/AU
L1	152	S E1-E3
		E PATHAN ANSAR/AU
L2	33	S E1-E5
L3	169	S L1-L2
L4	38	S L3 AND ESAT-6
L5	0	S L4 AND (ES1 OR ES2 OR ES3)
L6	12	DUP REM L4 (26 DUPLICATES REMOVED)
L7	583	S ESAT-6
L8	564	S L7 AND TUBERCULOSIS
L9	11	S L8 AND T CELL RECOGNI?
L10	3	DUP REM L9 (8 DUPLICATES REMOVED)
L11	35	S L8 AND T CELL (5A) RECOGNI?
L12	20	S L11 AND (DIAGNOSIS OR DIAGNOSTIC OR ASSAY OR DETECT?)
L13	7	DUP REM L12 (13 DUPLICATES REMOVED)
L14	382	S L8 AND VACCIN?
L15	69	S L14 AND (TREATING OR TREATMENT OR PREVENTING OR PREVENTION)
L16	47	DUP REM L15 (22 DUPLICATES REMOVED)

=> s l8 and epitop? (5a) mapping
L17 17 L8 AND EPITOP? (5A) MAPPING

the individual whether the level of T cells specific for a mycobacterial antigen has decreased after the treatment, thereby detg. the efficacy of the treatment.

L18 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2002:559608 BIOSIS
DN PREV200200559608
TI **Epitope mapping** of the immunodominant antigen TB10.4 and the two homologous proteins TB10.3 and TB12.9, which constitute a subfamily of the **esat-6** gene family.
AU Skjot, Rikke Louise Vinther; Brock, Inger; Arend, Sandra M.; Munk, Martin E.; Theisen, Michael; Ottenhoff, Tom H. M.; Andersen, Peter (1)
CS (1) Department of TB Immunology, Statens Serum Institut, Artillerivej 5, DK-2300, Copenhagen S: pa@ssi.dk Denmark
SO Infection and Immunity, (October, 2002) Vol. 70, No. 10, pp. 5446-5453. print.
ISSN: 0019-9567.
DT Article
LA English
AB The human T-cell recognition of the low-molecular-mass culture filtrate antigen TB10.4 was evaluated in detail. The molecule was strongly recognized by T cells isolated from **tuberculosis** (TB) patients and from BCG-vaccinated donors. The epitopes on TB10.4 were mapped with overlapping peptides and found to be distributed throughout the molecule. The broadest response was found in TB patients, whereas the response in BCG-vaccinated donors was focused mainly toward a dominant epitope located in the N terminus (amino acids 1 to 18). The gene encoding TB10.4 was found to belong to a subfamily within the **esat-6** family that consists of the three highly homologous proteins TB10.4, TB10.3, and TB12.9 (Rv0288, Rv3019c, and Rv3017c, respectively). Southern blot analysis combined with database searches revealed that the three members of the TB10.4 family were present only in strains of the Mycobacterium **tuberculosis** complex, including BCG, and M. kansasii, whereas other atypical mycobacteria had either one (M. avium, M. intracellulare, and M. marinum) or none (M. scrofulaceum, M. fortuitum, and M. szulgai) of the genes. The fine specificity of the T-cell response to the three closely related **esat-6** family members was markedly different, with only a few epitopes shared between the molecules. Minimal differences in the amino acid sequence translated into large differences in recognition by T cells and secretion of gamma interferon. In general, the peptides from TB10.4 stimulated the largest responses, but epitopes unique to both TB10.3 and TB12.9 were found. The relevance of the findings for TB vaccine development and as a potential mechanism for immune evasion is discussed.

L18 ANSWER 5 OF 8 MEDLINE
AN 2002724225 MEDLINE
DN 22328469 PubMed ID: 12441800
TI Rapid detection of active and latent **tuberculosis** infection in HIV-positive individuals by enumeration of Mycobacterium **tuberculosis**-specific T cells.
AU Chapman Ann L N; Munkanta Mwansa; Wilkinson Katalin A; Pathan Ansar A; Ewer Katie; Ayles Helen; Reece William H; Mwinga Alwyn; Godfrey-Faussett Peter; Lalvani Ajit
CS Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK.
SO AIDS, (2002 Nov 22) 16 (17) 2285-93.
Journal code: 8710219. ISSN: 0269-9370.
CY England: United Kingdom
DT (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS

mycobacteria. We recently identified circulating **ESAT-6** -specific T cells as an accurate marker of M. **tuberculosis** infection. Here, interferon-gamma-secreting T cells specific for peptides derived from **ESAT-6** and a second RD1 gene product, CFP10, were enumerated in 100 prospectively recruited healthy adults in Bombay (Mumbai), India. Eighty percent responded to >/=1 antigen, and many donors had high frequencies of T cells that were specific for certain immunodominant peptides. In contrast, of 40 mostly BCG-vaccinated, United Kingdom-resident healthy adults, none responded to either antigen. This study suggests an 80% prevalence of latent M. **tuberculosis** infection in urban India.

L18 ANSWER 7 OF 8 MEDLINE DUPLICATE 2
AN 2000417674 MEDLINE
DN 20336480 PubMed ID: 10875783
TI Multiple epitopes from the Mycobacterium **tuberculosis** **ESAT-6** antigen are recognized by antigen-specific human T cell lines.
AU Mustafa A S; Oftung F; Amoudy H A; Madi N M; Abal A T; Shaban F; Rosen Krands I; Andersen P
CS Department of Microbiology, Kuwait University, Safat 13110, Kuwait.. abusalim@hsc.kuniv.edu.kw
SO CLINICAL INFECTIOUS DISEASES, (2000 Jun) 30 Suppl 3 S201-5. Journal code: 9203213. ISSN: 1058-4838.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200009
ED Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000906
AB A synthetic-peptide approach was used to map epitope regions of the Mycobacterium **tuberculosis** 6-kDa early secreted antigen target (**ESAT-6**) by testing human CD4(+) T cell lines for secretion of IFN-gamma in response to recombinant **ESAT-6** (rESAT-6) and overlapping 20-mer peptides covering the antigen sequence. The results demonstrate that all of the **ESAT-6** peptides screened were able to induce IFN-gamma secretion from one or more of the T cell lines tested. Some of the individual T cell lines showed the capacity to respond to all peptides. Human leukocyte antigen (HLA-DR) typing of the donors showed that rESAT-6 was presented to T cells in association with multiple HLA-DR molecules. The results suggest that frequent recognition of the M. **tuberculosis** **ESAT-6** antigen by T cells from patients with **tuberculosis** is due to the presence of multiple epitopes scattered throughout the **ESAT-6** sequence.

L18 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1998:120633 BIOSIS
DN PREV199800120633
TI B-cell epitopes and quantification of the **ESAT-6** protein of Mycobacterium **tuberculosis**.
AU Harboe, Morten (1); Malin, Adam S.; Dockrell, Hazel S.; Wiker, Harald Gotten; Ulvund, Gunni; Holm, Arne; Jorgensen, Mikala Clok; Andersen, Peter
CS (1) Inst. Immunol. Rheumatol., Univ. Oslo, Fr. Qvams gate 1, N-0172 Oslo Norway
SO Infection and Immunity, (Feb., 1998) Vol. 66, No. 2, pp. 717-723. ISSN: 0019-9567.
DT Article
LA English
AB **ESAT-6** is an important T-cell antigen recognized by protective T cells in animal models of infection with Mycobacterium

tuberculosis. In an enzyme-linked immunosorbent assay (ELISA) with overlapping peptides spanning the sequence of **ESAT-6**, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the molecule. Assays with synthetic truncated peptides allowed a precise **mapping** of the **epitope** to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two additional areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native **ESAT-6**. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The assay was suitable for quantification of **ESAT-6** in M. **tuberculosis** antigen preparations, showing no reactivity with M. bovis BCG Tokyo culture fluid, used as a negative control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of **ESAT-6** expression from vaccinia virus constructs containing the **esat-6** gene; this expression could not be identified by standard immunoblotting.

```
=> s early secretory antigenic target?
L19         123 EARLY SECRETORY ANTIGENIC TARGET?

=> s l19 and tuberculosis
L20         120 L19 AND TUBERCULOSIS

=> s l20 and (treating or treatment or preventing or prevention)
L21          30 L20 AND (TREATING OR TREATMENT OR PREVENTING OR PREVENTION)

=> dup rem l21
PROCESSING COMPLETED FOR L21
L22           28 DUP REM L21 (2 DUPLICATES REMOVED)

=> d bib ab 1-28

L22  ANSWER 1 OF 28      MEDLINE
AN   2003204569      MEDLINE
DN   22610420    PubMed ID: 12692547
TI   Building a better tuberculosis vaccine.
CM   Comment on: Nat Med. 2003 May;9(5):533-9
AU   Young Douglas B
SO   NATURE MEDICINE, (2003 May) 9 (5) 503-4.
     Journal code: 9502015. ISSN: 1078-8956.
CY   United States
DT   Commentary
     News Announcement
LA   English
FS   Priority Journals
EM   200306
ED   Entered STN: 20030502
     Last Updated on STN: 20030627
     Entered Medline: 20030626

L22  ANSWER 2 OF 28      MEDLINE
AN   2003139729      MEDLINE
DN   22541561    PubMed ID: 12654848
TI   Enhanced murine antigen-specific gamma interferon and immunoglobulin G2a
     responses by using mycobacterial ESAT-6 sequences in DNA vaccines.
```

AU Minion F Chris; Menon Sreekumar A; Mahairas Gregory G; Wannemuehler M J
CS Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.. fcminion@iastate.edu
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 2239-43.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB The Mycobacterium **tuberculosis** protein ESAT-6 has unusual immune stimulating activities, has been implicated in the recall of long-lived immunity, and induces protection against **tuberculosis** in mice. For many diseases caused by bacterial or viral pathogens, a strong cell-mediated immune (i.e., type 1) response is often required for recovery or protection. Therefore, it is important to design immunization regimens that induce agent-specific type 1 immunity. We have shown in previous studies that ESAT-6 could enhance antigen-specific type 1 immune responses in BALB/c mice against a second antigen when presented as a purified fusion protein. It was also of interest to determine if ESAT-6 could enhance the type 1 response against a second antigen beyond that afforded by DNA vaccination through CpG motifs. This was tested by using gene fusions of ESAT-6 and the Mycoplasma hyopneumoniae surface antigen P71. Modified P71 gene sequences were cloned with or without ESAT-6 sequences into a DNA vaccine vector and were used to immunize mice. Splenic lymphocytes from vaccinated mice were tested for gamma interferon (IFN-gamma) and interleukin-10 (IL-10) secretion. Serum antibodies were examined for P71 antigen-specific isotype responses. When stimulated in vitro with purified P71 antigen, splenocytes from the ESAT-6:P71 vaccinates secreted higher levels of IFN-gamma and lower levels of IL-10 compared to those of vaccinates receiving the P71 construct alone. Furthermore, the immunoglobulin G2a serum antibody levels were significantly higher in the ESAT-6:P71 vaccinates compared to those of the vaccinates receiving P71 alone. In conclusion, ESAT-6 was shown to enhance antigen-specific type 1 immune responses in BALB/c mice when used in DNA vaccines.

L22 ANSWER 3 OF 28 MEDLINE
AN 2003139677 MEDLINE
DN 22541491 PubMed ID: 12654778
TI Virulence, immunogenicity, and protective efficacy of two recombinant Mycobacterium bovis bacillus Calmette-Guerin strains expressing the antigen ESAT-6 from Mycobacterium **tuberculosis**.
AU Bao Lang; Chen Wei; Zhang Huidong; Wang Xiaoying
CS Research Unit of Infection and Immunity, West China Medical Center, Sichuan University, No. 17, 3rd Section, Ren Min Nan Road, Chengdu, Sichuan 610041, People's Republic of China.. baolang@wcums.edu.cn
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 1656-61.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030326
Last Updated on STN: 20030513
Entered Medline: 20030512
AB We constructed two recombinant Mycobacterium bovis BCG (rBCG) strains expressing ESAT-6 of Mycobacterium **tuberculosis**, named rBCG-1 and rBCG-2. rBCG-1 contained the ESAT-6 gene linked to BCG hsp60 and

DN 21655153 PubMed ID: 11796598
TI Failure of the Mycobacterium bovis BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to **tuberculosis**.
AU Brandt Lise; Feino Cunha Joana; Weinreich Olsen Anja; Chilima Ben; Hirsch Penny; Appelberg Rui; Andersen Peter
CS Department of TB Immunology, Statens Serum Institut, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (2002 Feb) 70 (2) 672-8.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200202
ED Entered STN: 20020128
Last Updated on STN: 20030111
Entered Medline: 20020221
AB The efficacy of Mycobacterium bovis bacillus Calmette-Guerin (BCG) vaccine against pulmonary **tuberculosis** (TB) varies enormously in different populations. The prevailing hypothesis attributes this variation to interactions between the vaccine and mycobacteria common in the environment, but the precise mechanism has so far not been clarified. Our study demonstrates that prior exposure to live environmental mycobacteria can result in a broad immune response that is recalled rapidly after BCG vaccination and controls the multiplication of the vaccine. In these sensitized mice, BCG elicits only a transient immune response with a low frequency of mycobacterium-specific cells and no protective immunity against TB. In contrast, the efficacy of TB subunit vaccines was unaffected by prior exposure to environmental mycobacteria. Six different isolates from soil and sputum samples from Karonga district in Northern Malawi (a region in which BCG vaccination has no effect against pulmonary TB) were investigated in the mouse model, and two strains of the Mycobacterium avium complex were found to block BCG activity completely.

L22 ANSWER 11 OF 28 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2003192728 EMBASE
TI **Tuberculosis** vaccines: The past, present and future.
AU Wang J.; Xing Z.
CS Dr. Z. Xing, Department of Pathology, Centre for Gene Therapeutics, McMaster University, 1200 Main Street West, Hamilton, Ont. L8N 3Z5, Canada. xingz@mcmaster.ca
SO Expert Review of Vaccines, (2002) 1/3 (341-354).
Refs: 111
ISSN: 1476-0584 CODEN: ERVXAX
CY United Kingdom
DT Journal; General Review
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB **Tuberculosis** still remains a leading infectious cause of death worldwide, although the BCG vaccine has been used for 80 years. There is an urgent need to develop improved BCG or new **tuberculosis** vaccines. This apparently represents a daunting task, since it will take a long time before a vaccine can be declared to be better than the current BCG vaccine, both in experimental and human studies. The current review takes a brief historic look at the use of current BCG vaccine and provides an overview on what are considered to be the key Immunologic criteria that have to be met by a new generation of **tuberculosis** vaccines. It also provides the most up-to-date information on the latest developments

in **tuberculosis** vaccine research, with a focus on mycobacterial organism-based and Mycobacterium **tuberculosis** antigen-based vaccines. Consideration is also given to the mucosal route of immunization and 'prime and boost' regimens. This review also presents several important tables, highlighting critical components of antituberculosis immunity, the most commonly tested immune adjuvants, the types of novel **tuberculosis** antigen-based vaccines and the outcome of different heterologous 'prime and boost' vaccination regimens.

L22 ANSWER 12 OF 28 MEDLINE
AN 2001503905 MEDLINE
DN 21437669 PubMed ID: 11553606
TI **Tuberculosis** contacts but not patients have higher gamma interferon responses to ESAT-6 than do community controls in The Gambia.
AU Vekemans J; Lienhardt C; Sillah J S; Wheeler J G; Lahai G P; Doherty M T; Corrah T; Andersen P; McAdam K P; Marchant A
CS Medical Research Council Laboratories, Fajara, The Gambia.. Johan.Vekemans@ulb.ac.be
SO INFECTION AND IMMUNITY, (2001 Oct) 69 (10) 6554-7. Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20010913
Last Updated on STN: 20011029
Entered Medline: 20011025
AB The Mycobacterium **tuberculosis** antigen ESAT-6 has been proposed for **tuberculosis** immunodiagnosis. In The Gambia, 30% of community controls produced gamma interferon (IFN-gamma) in response to ESAT-6. Increased proportions of responders and intensities of responses were found in household contacts. Responses that were initially low in **tuberculosis** patients increased after **treatment**. An ESAT-6 IFN-gamma assay will be of limited use in the diagnosis of **tuberculosis** in countries where **tuberculosis** is endemic. Its role in contact tracing should be evaluated further.

L22 ANSWER 13 OF 28 MEDLINE
AN 2001567381 MEDLINE
DN 21528960 PubMed ID: 11673535
TI Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium **tuberculosis**-infected individuals: associations with clinical disease state and effect of **treatment**.
AU Pathan A A; Wilkinson K A; Klenerman P; McShane H; Davidson R N; Pasvol G; Hill A V; Lalvani A
CS Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.
SO JOURNAL OF IMMUNOLOGY, (2001 Nov 1) 167 (9) 5217-25. Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200112
ED Entered STN: 20011024
Last Updated on STN: 20020122
Entered Medline: 20011205
AB The wide spectrum of clinical outcomes following infection with Mycobacterium **tuberculosis** is largely determined by the host immune response; therefore, we studied several clinically defined groups of individuals (n = 120) that differ in their ability to contain the

absent from BCG constitute prime candidates for differential diagnostic reagents. Recently, two such antigens, ESAT-6 and CFP-10, have been reported to be promising candidates as diagnostic reagents for the detection of M. bovis infection in cattle. Here we report the identification of promiscuous peptides of CFP-10 that were recognized by M. bovis-infected cattle. Five of these peptides were formulated into a peptide cocktail together with five peptides derived from ESAT-6. Using this peptide cocktail in T-cell assays, M. bovis-infected animals were detected, while BCG-vaccinated or Mycobacterium avium-sensitized animals did not respond. The sensitivity of the peptide cocktail as an antigen in a whole-blood gamma interferon assay was determined using naturally infected field reactor cattle, and the specificity was determined using blood from BCG-vaccinated and noninfected, nonvaccinated animals. The sensitivity of the assay in cattle with confirmed **tuberculosis** was found to be 77.9%, with a specificity of 100% in BCG-vaccinated or nonvaccinated animals. This compares favorably with the specificity of tuberculin when tested in noninfected or vaccinated animals. In summary, our results demonstrate that this peptide cocktail can discriminate between M. bovis infection and BCG vaccination with a high degree of sensitivity and specificity.

L22 ANSWER 19 OF 28 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001259407 EMBASE
TI TB vaccines: Progress and problems.
AU Andersen P.
CS P. Andersen, Dept. of TB Immunology, Statens Seruminstitut, 5 Artillerivej, DK-2300 Copenhagen S, Denmark. pa@ssi.dk
SO Trends in Immunology, (2001) 22/3 (160-168).
Refs: 77
ISSN: 1471-4906 CODEN: TIRMAE
PUI S 1471-4906(01)01865-8
CY United Kingdom
DT Journal; General Review
FS 015 Chest Diseases, Thoracic Surgery and Tuberculosis
022 Human Genetics
026 Immunology, Serology and Transplantation
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB **Tuberculosis** (TB) is the biggest killer worldwide of any infectious disease, a situation worsened by the advent of the HIV epidemic and the emergence of multi-drug resistant strains of Mycobacterium **tuberculosis**. The existing vaccine, Mycobacterium bovis bacille Calmette-Guerin (BCG), has proven inefficient in several recent field trials. There is currently intense research using cutting-edge vaccine technology to combat this ancient disease. However, it is necessary to understand why BCG has failed before we can rationally develop the next generation of vaccines. Several hypotheses that might explain the failure of BCG and the strategies designed to address these shortcomings are discussed.

L22 ANSWER 20 OF 28 MEDLINE
AN 2000283769 MEDLINE
DN 20283769 PubMed ID: 10823800
TI Detection of active **tuberculosis** infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10.
CM Comment in: J Infect Dis. 2001 Dec 1;184(11):1497-8
AU Arend S M; Andersen P; van Meijgaarden K E; Skjot R L; Subronto Y W; van Dissel J T; Ottenhoff T H
CS Dept. of Infectious Diseases, C5P, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.. smarend@lumc.nl

SO JOURNAL OF INFECTIOUS DISEASES, (2000 May) 181 (5) 1850-4.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200007
ED Entered STN: 20000728
Last Updated on STN: 20030105
Entered Medline: 20000720
AB The purified protein derivative (PPD) skin test has no predictive value for **tuberculosis** (TB) in Mycobacterium bovis bacillus Calmette-Guerin (BCG)-vaccinated individuals because of cross-reactive responses to nonspecific constituents of PPD. T cell responses to early-secreted antigenic target 6-kDa protein (ESAT-6) and the newly identified culture filtrate protein 10 (CFP-10), 2 proteins specifically expressed by M. **tuberculosis** (MTB) but not by BCG strains, were evaluated. Most TB patients responded to ESAT-6 (92%) or CFP-10 (89%). A minority of BCG-vaccinated individuals responded to both ESAT-6 and CFP-10, their history being consistent with latent infection with MTB in the presence of protective immunity. No responses were found in PPD-negative controls. The sensitivity and specificity of the assay were 84% and 100%, respectively, at a cutoff of 300 pg of interferon-gamma/mL. These data indicate that ESAT-6 and CFP-10 are promising antigens for highly specific immunodiagnosis of TB, even in BCG-vaccinated individuals.

L22 ANSWER 21 OF 28 MEDLINE DUPLICATE 2
AN 2000386478 MEDLINE
DN 20354875 PubMed ID: 10898510
TI Efficient protection against Mycobacterium **tuberculosis** by vaccination with a single subdominant epitope from the ESAT-6 antigen.
AU Olsen A W; Hansen P R; Holm A; Andersen P
CS Department of TB Immunology, Statens Serum Institute, Copenhagen, Denmark.
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Jun) 30 (6) 1724-32.
Journal code: 1273201. ISSN: 0014-2980.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200008
ED Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000809
AB We have investigated the vaccine potential of two peptides derived from the 6-kDa **early secretory antigenic target** (ESAT)-6 antigen in the mouse model of **tuberculosis**. The peptides were both strongly immunogenic in B6CBAF1 (H-2b,k) mice and primed recall responses of the same intensity after immunization. However, both **tuberculosis** infection and immunization with ESAT-6 resulted in responses focused towards ESAT-61-20. Multiple antigen peptide constructs as well as free peptides were emulsified with dimethyl dioctadecylammonium bromide/monophosphoryl lipid A/IL-2 and tested as experimental vaccines in an i.v. and aerosol model of **tuberculosis** in mice. The peptide were highly immunogenic and induced cellular responses of the same magnitude. However, only vaccines based on the subdominant ESAT-651-70 epitope promoted significant levels of protective immunity and the level of protection was equivalent to that achieved with ESAT-6 and BCG. These findings demonstrate the potential of peptide-based vaccines against **tuberculosis** and indicate that there is not direct correlation between the hierarchy of response to naturally processed peptides and their ability to induce protective immunity against Mycobacterium **tuberculosis**.

L22 ANSWER 22 OF 28 MEDLINE
AN 2001129016 MEDLINE
DN 21017841 PubMed ID: 11144463
TI Numbers of IFN-gamma-producing cells against ESAT-6 increase in **tuberculosis** patients during chemotherapy.
AU Ulrichs T; Anding R; Kaufmann S H; Munk M E
CS Max-Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany.
SO INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE, (2000 Dec) 4 (12) 1181-3.
Journal code: 9706389. ISSN: 1027-3719.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200103
ED Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301
AB ESAT-6 is a specific Mycobacterium **tuberculosis** complex antigen and strong inducer of interferon-gamma (IFN-gamma) production by T cells from **tuberculosis** patient T-cells. We studied the frequency of IFN-gamma producing cells reacting to ESAT-6 during anti-**tuberculosis** chemotherapy. The numbers of IFN-gamma producing cells in the peripheral blood were higher in **tuberculosis** patients after discharge from specific anti-**tuberculosis** chemotherapy, compared with untreated patients. These results indicate that monitoring specific M. **tuberculosis** antigen reactivity during anti-**tuberculosis** chemotherapy may avoid premature termination of **treatment** and resistant strains.

L22 ANSWER 23 OF 28 MEDLINE
AN 2000417694 MEDLINE
DN 20336500 PubMed ID: 10875803
TI Toward the development of diagnostic assays to discriminate between Mycobacterium bovis infection and bacille Calmette-Guerin vaccination in cattle.
AU Vordermeier H M; Cockle P J; Whelan A O; Rhodes S; Hewinson R G
CS Tuberculosis Research Group, Bacteriology Department, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.. mvordermeier.vla@gtnet.gov.uk
SO CLINICAL INFECTIOUS DISEASES, (2000 Jun) 30 Suppl 3 S291-8.
Journal code: 9203213. ISSN: 1058-4838.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200009
ED Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000906
AB A scientific review of the recent sharp increase in bovine **tuberculosis** in Great Britain has concluded that the development of a cattle vaccine holds the best prospect for long-term disease control. It is important to develop a diagnostic test that differentiates between vaccinated and Mycobacterium bovis-infected animals, to ensure that test-and-slaughter control strategies can continue alongside vaccination. The mycobacterial antigens ESAT-6, MPB64, and MPB83 are expressed at high levels in M. bovis but are expressed at low levels or not at all in bacille Calmette-Guerin (BCG) Pasteur. Promiscuous bovine T cell epitopes of these antigens were identified and formulated into a peptide cocktail. This cocktail and a cocktail composed of recombinant forms of the 3 antigens was able to distinguish cattle infected with virulent M. bovis

from those vaccinated with BCG and from those sensitized to avian tuberculin in lymphocyte transformation and interferon-gamma assays.

L22 ANSWER 24 OF 28 MEDLINE
AN 2000072687 MEDLINE
DN 20072687 PubMed ID: 10603390
TI Comparative evaluation of low-molecular-mass proteins from Mycobacterium **tuberculosis** identifies members of the ESAT-6 family as immunodominant T-cell antigens.
AU Skjot R L; Oettinger T; Rosenkrands I; Ravn P; Brock I; Jacobsen S; Andersen P
CS Department of TB Immunology, Statens Serum Institut, Copenhagen, Denmark.
SO INFECTION AND IMMUNITY, (2000 Jan) 68 (1) 214-20.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
ED Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000111
AB Culture filtrate from Mycobacterium **tuberculosis** contains protective antigens of relevance for the generation of a new antituberculosis vaccine. We have identified two previously uncharacterized M. **tuberculosis** proteins (TB7.3 and TB10.4) from the highly active low-mass fraction of culture filtrate. The molecules were characterized, mapped in a two-dimensional electrophoresis reference map of short-term culture filtrate, and compared with another recently identified low-mass protein, CFP10 (F. X. Berthet, P. B. Rasmussen, I. Rosenkrands, P. Andersen, and B. Gicquel. Microbiology 144:3195-3203, 1998), and the well-described ESAT-6 antigen. Genetic analyses demonstrated that TB10.4 as well as CFP10 belongs to the ESAT-6 family of low-mass proteins, whereas TB7.3 is a low-molecular-mass protein outside this family. The proteins were expressed in Escherichia coli, and their immunogenicity was tested in cultures of peripheral blood mononuclear cells from human **tuberculosis** (TB) patients, Mycobacterium bovis BCG-vaccinated donors, and nonvaccinated donors. The two ESAT-6 family members, TB10.4 and CFP10, were very strongly recognized and induced gamma interferon release at the same level (CFP10) as or at an even higher level (TB10.4) than ESAT-6. The non-ESAT-6 family member, TB7.3, for comparison, was recognized at a much lower level. CFP10 was found to distinguish TB patients from BCG-vaccinated donors and is, together with ESAT-6, an interesting candidate for the diagnosis of TB. The striking immunodominance of antigens within the ESAT-6 family is discussed, and hypotheses are presented to explain this targeting of the immune response during TB infection.

L22 ANSWER 25 OF 28 MEDLINE
AN 2001063036 MEDLINE
DN 20507278 PubMed ID: 11052907
TI An esat6 knockout mutant of Mycobacterium bovis produced by homologous recombination will contribute to the development of a live **tuberculosis** vaccine.
AU Wards B J; de Lisle G W; Collins D M
CS Wallaceville Animal Research Centre, AgResearch, Upper Hutt, New Zealand.. wardsb@agresearch.cri.nz
SO TUBERCLE AND LUNG DISEASE, (2000) 80 (4-5) 185-9.
Journal code: 9212467. ISSN: 0962-8479.
CY SCOTLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

Last Updated on STN: 19980224
Entered Medline: 19980212

AB ESAT-6 is an important T-cell antigen recognized by protective T cells in animal models of infection with Mycobacterium **tuberculosis**. In an enzyme-linked immunosorbent assay (ELISA) with overlapping peptides spanning the sequence of ESAT-6, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the molecule. Assays with synthetic truncated **peptides** allowed a precise **mapping** of the epitope to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two additional areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native ESAT-6. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The assay was suitable for quantification of ESAT-6 in M. **tuberculosis** antigen preparations, showing no reactivity with M. bovis BCG Tokyo culture fluid, used as a negative control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of ESAT-6 expression from vaccinia virus constructs containing the esat-6 gene; this expression could not be identified by standard immunoblotting.

L25 ANSWER 2 OF 3 MEDLINE
AN 97025462 MEDLINE
DN 97025462 PubMed ID: 8871652
TI Key epitopes on the ESAT-6 antigen recognized in mice during the recall of protective immunity to Mycobacterium **tuberculosis**.
AU Brandt L; Oettinger T; Holm A; Andersen A B; Andersen P
CS The TB Research Unit, Bacterial Vaccine Department, Statens Seruminstitut, Copenhagen, Denmark.
SO JOURNAL OF IMMUNOLOGY, (1996 Oct 15) 157 (8) 3527-33.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961217

AB The recall of long-lived immunity in a mouse model of **tuberculosis** (TB) is defined as an accelerated accumulation of reactive T cells in the target organs. We have recently identified Ag 85B and a 6-kilodalton **early secretory antigenic target**, designated ESAT-6, as key antigenic targets recognized by these cells. In the present study, preferential recognition of the ESAT-6 Ag during the recall of immunity was found to be shared by five of six genetically different strains of mice. Overlapping peptides spanning the sequence of ESAT-6 were used to map two T cell epitopes on this molecule. One epitope recognized in the context of H-2b,d was located in the N-terminal part of the molecule, whereas an epitope recognized in the context of H-2a,k covered amino acids 51 to 60. Shorter versions of the N-terminal epitope allowed the precise definition of a 13-amino acid core sequence recognized in the context of H-2b. The peptide covering the N-terminal epitope was immunogenic, and a T cell response with the same fine specificity as that induced during TB infection was generated by immunization with the peptide in IFA. In the C57BL/6j strain, this single epitope was recognized by an exceedingly high frequency of splenic T cells (approximately 1:1000), representing 25 to 35% of the total culture filtrate-reactive T cells

recruited to the site of infection during the first phase of the recall response. These findings emphasize the relevance of this Ag in the immune response to TB and suggest that immunologic recognition in the first phase of infection is a highly restricted event dominated by a limited number of T cell clones.

L25 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS
AN 1998:67789 CAPLUS
DN 128:179092
TI B-cell epitopes and quantification of the ESAT-6 protein of Mycobacterium **tuberculosis**
AU Harboe, Morten; Malin, Adam S.; Dockrell, Hazel S.; Wiker, Harald Gotten; Ulvund, Gunni; Holm, Arne; Jorgensen, Mikala Clok; Andersen, Peter
CS Institute of Immunology and Rheumatology, University of Oslo, Oslo, N-0172, Norway
SO Infection and Immunity (1998), 66(2), 717-723
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB ESAT-6 is an important T-cell antigen recognized by protective T cells in animal models of infection with Mycobacterium **tuberculosis**. In an ELISA with overlapping peptides spanning the sequence of ESAT-6, monoclonal antibody HYB76-8 reacted with two peptides in the N-terminal region of the mol. Assays with synthetic truncated **peptides** allowed a precise **mapping** of the epitope to the residues EQQWNFAGIEAAA at positions 3 to 15. Hydrophilicity plots revealed one hydrophilic area at the N terminus and two addnl. areas further along the polypeptide chain. Antipeptide antibodies were generated by immunization with synthetic 8-mer peptides corresponding to these two regions coupled to keyhole limpet hemocyanin. Prolonged immunization with a 23-mer peptide (positions 40 to 62) resulted in the formation of antibodies reacting with the peptide as well as native ESAT-6. A double-antibody ELISA was then developed with monoclonal antibody HYB76-8 as a capture antibody, antigen for testing in the second layer, and antipeptide antibody in the third layer. The assay was suitable for quantification of ESAT-6 in M. **tuberculosis** antigen prepns., showing no reactivity with M. bovis BCG Tokyo culture fluid, used as a neg. control, or with MPT64 or antigen 85B, previously shown to cross-react with HYB76-8. This capture ELISA permitted the identification of ESAT-6 expression from vaccinia virus constructs contg. the esat-6 gene; this expression could not be identified by std. immunoblotting.
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s esat-6 and peptide? (5a) mapping
L26 9 ESAT-6 AND PEPTIDE? (5A) MAPPING

=> dup rem l26
PROCESSING COMPLETED FOR L26
L27 3 DUP REM L26 (6 DUPLICATES REMOVED)

=> d bib ab 1-3

L27 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2003:103124 BIOSIS
DN PREV200300103124
TI Proteome analysis of the plasma membrane of Mycobacterium tuberculosis.
AU Sinha, Sudhir (1); Arora, Shalini; Kosalai, K.; Namane, Abdelkader; Pym, Alex S.; Cole, Stewart T.
CS (1) Division of Biochemistry, Central Drug Research Institute, PO Box 173, Lucknow, 226001, India: sinhas@lycos.com India

Compositional analysis demonstrated that the protein was rich in proline and that mannose, galactose, glucose, and arabinose together represented about 4% of the total mass. The 45-kDa glycoprotein was subjected to proteolytic digestion with either the Asp-N or the Glu-C endopeptidase or subtilisin, peptides were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and glycopeptides were identified by reaction with concanavalin A. Peptides were further separated, and when they were analyzed by liquid chromatography-electrospray mass spectrometry for neutral losses of hexoses (162 mass units), four peptides were identified, indicating that these were glycosylated with hexose residues. One peptide, with an average molecular mass of 1,516 atomic mass units (AMU), exhibited a loss of two hexose units. The N-terminal sequence of the 1,516-AMU glycopeptide was determined to be DPEPAPPVP, which was identical to the sequence of the amino terminus of the mature protein, DPEPAP PVPXTA. Furthermore, analysis of the glycopeptide by secondary ion mass spectrometry demonstrated that the complete sequence of the glycopeptide was DPEPAPPVPTTA. From this, it was determined that the 10th amino acid, threonine, was O-glycosidically linked to a disaccharide composed of two hexose residues, probably mannose. This report establishes that true, O-glycosylated proteins exist in mycobacteria.

L30 ANSWER 16 OF 30 MEDLINE
AN 96163834 MEDLINE
DN 96163834 PubMed ID: 8590566
TI T-cell recognition of mycobacterial antigens.
AU Vordermeier H M
CS MRC Tuberculosis & Related Infectious Unit, Clinical Sciences Centre, Hammersmith Hospital, London, UK.
SO EUROPEAN RESPIRATORY JOURNAL. SUPPLEMENT, (1995 Sep) 20 657s-667s. Ref: 121
Journal code: 8910681. ISSN: 0904-1850.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; AIDS
EM 199604
ED Entered STN: 19960418
Last Updated on STN: 19990129
Entered Medline: 19960401
AB T-cells play a dominant role in the immune response to mycobacterial infections. Most recognized mycobacterial antigens have been identified by monoclonal antibody techniques and, subsequently, sequenced and isolated by molecular cloning. Both CD4+ and CD8+ alpha beta T-cells, as well as gamma delta T-cells have been shown to participate in anti-mycobacterial host responses. The antigens recognized by CD4+ T-cells have been studied in most detail, with particular interest on proteins actively secreted by tubercle bacilli, on lipoproteins and on heat shock or stress proteins. **Peptide mapping** of T-cell epitopes of several mycobacterial proteins has suggested that many of their epitopes are recognized permissively in the context of multiple human and mouse major histocompatibility complex (MHC) class II alleles. This finding is encouraging for the development of subunit vaccines and diagnostic reagents.

L30 ANSWER 17 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 1995:79775 BIOSIS
DN PREV199598094075
TI **Peptide mapping** of bovine T-cell epitopes for the 38 kDa **tuberculosis** antigen.
AU Pollock, J. M. (1); Douglas, A. J.; Mackie, D. P.; Neill, S. D.

albumin to form complexes with various metabolic products in pathol. conditions (diabetes, **tuberculosis**, thyrotoxicosis).

=> s l30 and esat-6
L31 2 L30 AND ESAT-6

=> d bib ab 1-2

L31 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:103124 BIOSIS
DN PREV200300103124
TI Proteome analysis of the plasma membrane of Mycobacterium **tuberculosis**.
AU Sinha, Sudhir (1); Arora, Shalini; Kosalai, K.; Namane, Abdelkader; Pym, Alex S.; Cole, Stewart T.
CS (1) Division of Biochemistry, Central Drug Research Institute, PO Box 173, Lucknow, 226001, India: sinhas@lycos.com India
SO Comparative and Functional Genomics, (December 2002, 2002) Vol. 3, No. 6, pp. 470-483. print.
ISSN: 1531-6912.
DT Article
LA English
AB The plasma membrane of Mycobacterium **tuberculosis** is likely to contain proteins that could serve as novel drug targets, diagnostic probes or even components of a vaccine against **tuberculosis**. With this in mind, we have undertaken proteome analysis of the membrane of M. **tuberculosis** H37Rv. Isolated membrane vesicles were extracted with either a detergent (Triton X114) or an alkaline buffer (carbonate) following two of the protocols recommended for membrane protein enrichment. Proteins were resolved by 2D-GE using immobilized pH gradient (IPG) strips, and identified by **peptide** mass **mapping** utilizing the M. **tuberculosis** genome database. The two extraction procedures yielded patterns with minimal overlap. Only two proteins, both HSPs, showed a common presence. MALDI-MS analysis of 61 spots led to the identification of 32 proteins, 17 of which were new to the M. **tuberculosis** proteome database. We classified 19 of the identified proteins as 'membrane-associated'; 14 of these were further classified as 'membrane-bound', three of which were lipoproteins. The remaining proteins included four heat-shock proteins and several enzymes involved in energy or lipid metabolism. Extraction with Triton X114 was found to be more effective than carbonate for detecting 'putative' M. **tuberculosis** membrane proteins. The protocol was also found to be suitable for comparing BCG and M. **tuberculosis** membranes, identifying **ESAT-6** as being expressed selectively in M. **tuberculosis**. While this study demonstrates for the first time some of the membrane proteins of M. **tuberculosis**, it also underscores the problems associated with proteomic analysis of a complex membrane such as that of a mycobacterium.

L31 ANSWER 2 OF 2 MEDLINE
AN 97025462 MEDLINE
DN 97025462 PubMed ID: 8871652
TI Key epitopes on the **ESAT-6** antigen recognized in mice during the recall of protective immunity to Mycobacterium **tuberculosis**.
AU Brandt L; Oettinger T; Holm A; Andersen A B; Andersen P
CS The TB Research Unit, Bacterial Vaccine Department, Statens Seruminstitut, Copenhagen, Denmark.
SO JOURNAL OF IMMUNOLOGY, (1996 Oct 15) 157 (8) 3527-33.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)